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Research report

Neuroanatomical correlates of olfactory loss in normal aged subjects

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HIGHLIGHTS

- Olfactory loss is associated in normal aged subjects with gray and white matter brain changes.
- DTI shows microstructural changes in superior longitudinal fasciculi and corpus callosum.
- VBM shows GM volume loss in entorhinal and perirhinal olfactory structures.

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ABSTRACT

In non-demented older persons, smell dysfunction, measured premortem, has been associated with postmortem brain degeneration similar to that of Alzheimer's disease. We hypothesized that distinct measures of gray and white matter integrity evaluated through magnetic resonance imaging (MRI) techniques could detect degenerative changes associated with age-related olfactory dysfunction. High-resolution T1-weighted images and diffusion-tensor images (DTI) of 30 clinically healthy subjects aged 51–77 were acquired with a 3-Tesla MRI scanner. Odor identification performance was assessed by means of the University of Pennsylvania Smell Identification Test (UPSIT). UPSIT scores correlated with right amygdalar volume and bilateral perirhinal and entorhinal cortices gray matter volume. Olfactory performance also correlated with postcentral gyrus cortical thickness and with fractional anisotropy and mean diffusivity levels in the splenium of the corpus callosum and the superior longitudinal fasciculi. Our results suggest that age-related olfactory loss is accompanied by diffuse degenerative changes that might correspond to the preclinical stages of neurodegenerative processes.

1. Introduction

There is a large body of evidence linking olfactory loss and neurodegenerative processes. Olfactory impairments are strongly associated with Parkinson's (PD) and Alzheimer's (AD) diseases [1,2], and have been investigated in animal models of AD [3].

In normal aging, the association between olfactory impairment and brain degeneration has been reported in a longitudinal clinicopathological study that included a large cohort of 471 elderly subjects (*Rush Memory and Aging Project*). During a mean follow-up of 2.2 years, autopsies were obtained from 122 of 166 subjects who died, revealing that scores in odor identification correlated with neuropathological changes usually associated with AD: density of neurofibrillary tangles in the entorhinal cortex, in the CA1 subfield of the hippocampus and in the subiculum [4].

In a 5-year follow-up study including subjects from the same cohort, initial smell identification test scores were found to be associated with the risk of developing mild cognitive impairment (MCI). Moreover, in 34 subjects with olfactory dysfunction who died without cognitive deficits, autopsy showed greater burden of AD pathology [5].

The relationship between olfactory impairment and progressive cognitive decline was also seen in an epidemiological study involving 1920 participants with a mean age of 66.9 years. In this study, authors reported an association between olfactory impairment and the incidence of MCI 5 years later with an odds-ratio of 6.62 [6].

Magnetic resonance imaging (MRI) is an invaluable tool for the study of "in vivo" brain correlates of olfactory dysfunctions. In PD, olfactory impairment has been found to be related to white matter integrity loss detected by MRI diffusion-tensor imaging (DTI) [7]. On the other hand, voxel-based morphometry (VBM) studies have evidenced that subjects with hyposmia and anosmia of different

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etiology show gray and white matter reductions in central regions involved in the olfactory system [8,9].

The purpose of the current study was to investigate the cerebral correlates of impairments in odor identification in a sample of clinically healthy subjects and to correlate the performance in this task with MRI markers of cerebral degeneration such as cortical thickness, gray matter volumes and measures of white matter integrity. We hypothesized that olfactory dysfunctions in older persons could be associated with brain olfactory regions but also with other brain regions sensitive to the preclinical stages of degenerative processes.

2. Methods

2.1. Subjects

The sample included 30 clinically healthy subjects (12 males; mean age: 66.0 ± 7.4 years, range, 51–77 years; mean years of education: 11.1 ± 4.2). All were right handed. All the participants were recruited from healthy individuals who had volunteered to take part in studies addressing age-related processes at the Institut de l'Envelliment. The subjects received no monetary compensation for taking part in the study.

General exclusion criteria were: uncorrected visual or auditory deficits and history of past or current psychiatric or neurologic disorder or drug abuse. Specific exclusion criteria for the olfaction test were: history of nasal bone fracture, diagnosis of rhinitis or nasal polyps, and upper respiratory tract infections in the 2 weeks prior to or at the moment of evaluation. Imaging exclusion criteria included any abnormality except mild white matter hyperintensities. All selected participants completed a screening interview to review relevant medical information. One subject was currently a smoker, 7 had been smokers in the past and 22 had no history of smoking.

All subjects had normal general cognitive performance according to the Mini-Mental State Examination (scores ≥ 26) and normal global IQ scores (higher than 85) estimated by the vocabulary subtest of the Wechsler Adult Intelligence Scale-III [10]. All participants underwent a comprehensive neuropsychological assessment.

The study was approved by the ethics committee of the University of Barcelona. All enrolled subjects signed a written informed consent form before taking part in the study.

2.2. Olfactory assessment

Odor identification was assessed using the Spanish version of the University of Pennsylvania Smell Identification Test (UPSIT) [11]. The UPSIT is a standardized forced-choice test comprised of four booklets containing 10 odorants apiece, 1 odorant per page. The stimuli are embedded in "scratch and sniff" microcapsules fixed and positioned on strips at the bottom of each page. A multiple-choice question with four response alternatives for each item is located above each odorant strip. Scores are calculated as the number of items correctly identified. Respondents can be placed into percentiles based on gender- and age-standardized norms for the number of correctly identified odorants. The UPSITs are packaged in envelopes and come with easy-to-follow instructions. Following normative data presented in the UPSIT manual, scores greater than 33 were considered to reflect normosmia and scores lower than 19 were classified as anosmia. Scores between 19 and 32 reflected microsmia (from 19 to 25: severe microsmia; from 26 to 29: moderate microsmia and from 30 to 33: mild microsmia).

2.3. Neuropsychological assessment

We selected a neuropsychological battery including tests described as sensitive to aging effects and which have been found to be altered in the preclinical stages of Alzheimer's disease. The battery comprised tests measuring episodic memory (Rey Auditory Verbal Learning Test (RAVLT)), visuospatial and visuoperceptual functions (Benton's Judgment of Line Orientation and Facial Recognition) and executive functions (Trail Making Test, Stroop Color-Word Test and 1 min of phonetic (letters beginning with the letter P) and semantic (animals) fluencies). The characteristics of all tests are described in Lezak et al. [10]. Neuropsychological test results were analyzed using PASW-18 (Chicago, IL, http://www-01.ibm.com/software/analytics/spss/). Group differences in neuropsychological performance were tested using 3-level (normosmia, mild microsmia, moderate microsmia) one-way ANOVAs.

2.4. Image acquisition and analysis

Magnetic resonance images were acquired with a 3 T scanner (MAGNETOM Trio, Siemens, Germany). High-resolution 3-dimensional T1-weighted images were acquired in the sagittal plane (TR 2300 ms, TE 2.98 ms, TI 900 ms; 256 × 256 matrix, 1 mm isotropic voxel). Sagittal diffusion tensor images were obtained using a single-shot EPI sequence (TR 7700 ms, TE 89 ms), with diffusion-encoding in 30 directions at $b = 0$ and 1000 s/mm^2.

Structural data was analyzed with FSL-VBM [12], a voxel-based morphometry-style analysis carried out with FSL tools (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSL). First, nonbrain tissue from structural images was extracted. After segmentation, GM images were aligned to MNI152 standard space using affine registration. The resulting images were averaged to create a study-specific template, to which the native GM images were then non-linearly re-registered. The registered partial volume images were then modulated (to correct for local expansion or contraction) by dividing by the Jacobian of the warp field. The modulated segmented images were then smoothed with an isotropic Gaussian kernel with a sigma of 3 mm.

Voxelwise statistical analysis of fractional anisotropy (FA) and mean diffusivity (MD) data was carried out using TBSS (Tract-Based Spatial Statistics, [13]), part of FSL. First, FA and MD images were created by fitting a tensor model to the raw diffusion data using FDT, and then brain-extracted using BET [14]. All subjects' FA and MD data were then aligned into a common space using the nonlinear registration tool FNIRT (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FNIRT) which uses a b-spline representation of the registration warp field [15]. Next, mean FA and MD images were created and thinned to create mean FA and MD skeletons which represent the centers of all tracts common to the group. Each subject's aligned FA and MD data were then projected onto this skeleton and the resulting data fed into voxelwise cross-subject statistics.

Structures used as regions of interest (ROIs) for GM analysis were chosen based on literature about the olfactory system. The included structures were the amygdala, hippocampus, parahippocampal gyrus (encompassing entorhinal and perirhinal areas), olfactory portion of the orbitofrontal cortex, gyrus rectus and insula bilaterally. The Automated Anatomical Labeling (AAL) atlas [16] was used to create the corresponding masks.

Finally, voxelwise general linear model was applied using permutation-based non-parametric testing (5000 permutations) for FA, MD and VBM analyses, correcting for multiple comparisons across space using familywise-error correction (FWE).

The estimation of cortical thickness was performed using the automated FreeSurfer stream (version 5.1; available at: http://surfer.nmr.harvard.edu). The procedures carried out by FreeSurfer include removal of non-brain data, intensity normalization [17], tessellation of the gray matter/white matter boundary, automated topology correction [18,19] and accurate surface deformation to identify tissue borders [20–22]. Cortical thickness is then calculated as the distance between the white and gray matter surfaces at each vertex of the reconstructed cortical mantle [21]. Results for each subject were visually inspected to ensure accuracy of registration, skull stripping, segmentation, and cortical surface reconstruction.

The relationship between cortical thickness and UPSIT scores was assessed using a vertex-by-vertex general lineal model. Maps were smoothed using a circularly symmetric Gaussian kernel across the surface with a full width at half maximum (FWHM) of 15 mm. *Z* Monte Carlo simulations with 10,000 iterations were applied to CTh maps to provide cluster-wise correction for multiple comparisons, and results were thresholded at a corrected p value of 0.05 ($Z = 1.3$).

The automated procedure for volumetric measures of brain structures implemented in FreeSurfer was used to obtain the volumes of subcortical structures. Partial correlation between subcortical regions of interest (bilateral hippocampus and amygdala) and UPSIT scores, controlling for intracranial volume, were calculated using PASW-18.

3. Results

UPSIT scores ranged from 26 to 37 (mean 31.27, SD 2.95). Seven subjects (23.3%) were classified as normosmics; 15 (50%) had mild microsmia, 8 (26.7%) had moderate microsmia and none had severe microsmia or anosmia. There were no significant differences between these groups' neuropsychological test results (Table 1).

UPSIT scores did not correlate significantly with age ($r = -0.124$, $p = 0.513$), and did not differ significantly between males and females ($t = 0.09$, $p = 0.920$). For this reason, age and gender were not entered as covariates in the MRI models.

UPSIT scores correlated positively with cortical thickness in the right postcentral gyrus, indicating that olfactory dysfunction was associated with cortical thinning in this region ($Z = 3.128$; $p < 0.05$, Monte Carlo correction; cluster size: 1378.59 mm^2; x, y, z Talairach coordinates of the maximum: 48.3, −14.7, 42.3) (Fig. 1).

Whole-brain VBM analyses did not show any significant correlations at the corrected level. On the other hand, region-of-interest VBM analysis revealed a significant positive correlation between UPSIT scores and bilateral parahippocampal (entorhinal and perirhinal cortices) gray matter volumes. Left-side cluster: $r = 0.62$; $p = .017$, FWE-correction; cluster volume: 776 mm^3; x, y, z MNI coordinates of the maximum: −26, −10, −34. Right-side