Patterns of Brain Atrophy That Differentiate Corticobasal Degeneration Syndrome From Progressive Supranuclear Palsy

Adam L. Boxer, MD, PhD; Michael D. Geschwind, MD, PhD; Nataliya Belfor, PhD; Maria Luisa Gorno-Tempini, MD, PhD; Guido F. Schauer, BSc; Bruce L. Miller, MD; Michael W. Weiner, MD; Howard J. Rosen, MD

Background: Progressive brain atrophy is associated with the corticobasal degeneration syndrome (CBD) and progressive supranuclear palsy (PSP). Regional differences in brain atrophy may reflect the clinical features of disease.

Objective: To quantify the structural neuroanatomical differences between CBDS and PSP.

Design: A survey of neurologic deficits was conducted in all patients. Voxel-based morphometry was used to quantify structural neuroanatomical differences on magnetic resonance images in each subject group.

Setting: University hospital dementia clinic.

Participants: Fourteen patients who met clinical research criteria for CBD and 15 patients who met clinical research criteria for PSP, who were matched for severity of disease, age, and functional status, and 80 age-matched control subjects.

Main Outcome Measures: Statistically significant differences in regional gray and white matter volume, after multiple comparisons correction, between groups of subjects.

Results: The patients with CBDS displayed an asymmetric (left > right) pattern of brain atrophy that involved the bilateral premotor cortex, superior parietal lobules, and striatum. Progressive supranuclear palsy was associated with atrophy of the midbrain, pons, thalamus, and striatum, with minimal involvement of the frontal cortex. Midbrain structures were more atrophied in PSP than in CBD, whereas dorsal frontal and parietal cortices were more atrophied in CBD than in PSP. The degree of atrophy of the midbrain and ponsine tegmentum and the left frontal eye field differentiated the 2 patient groups with 93% accuracy.

Conclusions: Distinct patterns of brain atrophy exist in CBDS and PSP that can be used to differentiate the 2 diseases. Assessments of brain atrophy in these disorders should be focused on cortical and brainstem ocular motor control areas.
is severely impaired by CBDS and PSP, and quantitative eye movement measurements suggest that abnormalities in the velocity and latency of saccades can differentiate clinically defined cases of PSP from CBDS. The pattern of histopathologic findings in PSP has led to the hypothesis that damage to structures within the dorsal midbrain underlies the early and prominent vertical saccade slowing in PSP. In contrast, studies of patients with cortical lesions suggest that the increased latency of saccades in CBDS reflects damage to cortical eye fields. On the basis of these findings, we hypothesized that damage to cortical and brainstem ocular motor control regions would be measurable in living patients with CBDS and PSP as atrophy of the frontal eye fields in CBDS and the dorsal midbrain and pons in PSP. To test this hypothesis, we used voxel-based morphometry (VBM), an unbiased quantitative MRI analysis method with higher spatial resolution than the methods previously used, to compare regional atrophy in CBD and PSP.

STUDY PARTICIPANTS

A total of 109 individuals participated in the study: 80 control subjects, 15 patients with PSP, and 14 patients with CBDS. Informed consent was obtained for all components of the study. All PSP patients met the National Institute of Neurological Disorders and Stroke–Society for Progressive Supranuclear Palsy criteria for probable PSP. Briefly, these include (1) a gradually progressive disorder with onset at the age of 40 years or later and (2) vertical supranuclear gaze palsy and prominent postural instability within the first year of disease onset. The following features were required for a diagnosis of probable CBDS: (1) a slowly progressive course, (2) asymmetric limb or axial rigidity, present without reinforcement, (3) aphasia, visuospatial impairment or neglect, or apraxia, and (4) dystonia, myoclonus, cortical sensory loss, or alien limb phenomenon. All participants underwent MRI. The MRIs were not used in diagnostic decision making for definition of groups.

CLINICAL RATINGS

Patients’ medical records were reviewed by one of the authors (A.L.B). Neurologists’ spontaneous reports of clinical findings were recorded as present if mentioned in a clinic medical record up to and/or within 3 months of the MRI. All patients were rated on the Clinical Dementia Rating scale,11 the modified Hoehn and Yahr scale,12 and the Schwaab and England Activities of Daily Living Scale. The Hoehn and Yahr scale and Schwaab and England scale scores were calculated based on detailed neurologists’ reports from the patients’ clinical medical records. General intellectual function was assessed using the Mini-Mental State Examination (MMSE).14

MAGNETIC RESONANCE IMAGING

Images were obtained at the San Francisco Veterans Affairs Magnetic Resonance Unit. The T1-weighted (magnetization-prepared rapid gradient echo) MRIs were obtained on a 1.5-T Magnetom VISION system (Siemens Inc, Iselin, NJ). The structural MRI sequence used in our study was identical to that described in a previous study.

VOXEL-BASED MORPHOMETRY

Images were preprocessed and statistically analyzed with the SPM2 software package (http://www.fil.ion.ucl.ac.uk/spm), using standard procedures. To improve image spatial preprocessing, the “optimized” 2-step procedure was applied. A customized gray matter template, constructed by normalizing segmented gray matter images from 40 of the healthy control images (mean age, 66 years; 20 men and 20 women; 30 with full neurologic and neuropsychological evaluations) to the SPM2 gray matter template, was used for spatial normalization. Gray and white matter voxel values were multiplied by the jacobian determinants derived from the spatial normalization step to preserve the initial volumes. Images were spatially smoothed with a 12-mm full width at half maximum isotropic gaussian kernel. Total intracranial volume was used as a confounding covariate in an analysis of covariance. Age and sex for each study participant were entered into the design matrix as nuisance variables. A statistical threshold of P<.05 at the voxel level, corrected for multiple comparisons (familywise error), was accepted for the main contrasts.

To examine the patterns of atrophy specific to each patient group, the following contrasts were performed: (1) PSP patients vs controls: the areas of gray and white matter loss in the PSP patients relative to the controls were examined (PSP<controls); (2) CBDS patients vs controls: the areas of gray and white matter loss in the CBDS patients relative to the controls were examined (CBDS patients<controls); (3) PSP patients vs CBDS patients: the areas of gray and white matter loss in the PSP patients relative to the CBDS patients were examined (PSP patients<CBDS patients); (4) CBDS patients vs PSP patients: the areas of gray matter and loss in the CBDS patients relative to the PSP patients were examined (CBDS patients<PSP patients).

Localization of areas of significant cortical and subcortical gray matter loss was accomplished by superimposing the regions of significant atrophy on the averaged T1-weighted image used to create the template for spatial normalization and visual comparison with the cerebral atlases. Regions of atrophy are reported in Montreal Neurological Institute coordinates.

PATIENT CLASSIFICATION

Gray and white matter concentrations from voxels found to be most significantly different among groups in any of the 4 VBM contrasts were entered into a stepwise discriminant function analysis to identify which regions of atrophy best discriminated between the 2 patient groups. Statistical analysis was accomplished using the SPSS software package (version 10.0.5 for Windows, SPSS Inc, Chicago, Ill.).

RESULTS

CLINICAL FINDINGS

No differences were identified in age, education, or disease duration between the 2 patient groups (Table 1). There were more men than women in the PSP group but not in the CBD or control group. The CBD group was more impaired on the MMSE than the PSP group (P = .05; 2-tailed t test). Despite the difference in MMSE score, no difference was identified in functional status between the 2 patient groups on the Clinical Dementia Rating, the Hoehn and Yahr scale, and the Schwaab and England functional scales (Table 2).
Dystonia (P = .001), limb apraxia (P < .001), apraxia of speech (P = .004), myoclonus (P = .02), and cortical sensory deficits (P < .001) were present in a greater number of the CBD patients than PSP patients (χ² test; Table 3). Slow saccades (P = .001), vertical gaze limitation (P < .001), square wave jerks (P = .001), axial rigidity (P < .001), and falls (P = .001) were reported more frequently in the PSP group than in the CBD group. No significant difference were identified in the prevalence of limb rigidity, alien limb phenomenon, tremor, dysarthria, and subjective response to levodopa between the 2 patient groups.

NEUROIMAGING ANALYSIS OF REGIONAL BRAIN ATROPHY

CBD Patients vs Controls

Multiple regions of gray and white matter loss were identified in the frontal and parietal cortex and the basal ganglia when the 14 CBDs were compared with the 80 control subjects using VBM (Figure 1A, Table 4). There was an asymmetric pattern of cortical atrophy, with greater left-sided than right-sided involvement (Figure 1A, row 1). Bilaterally, the CBDs had volume loss relative to controls in the premotor cortex (Figure 1A, red) and frontal subcortical white matter (Figure 1A, yellow). Within this region, the most significant area of atrophy was bilaterally at the junction of the superior frontal sulci and the precentral sulci (Figure 1A, row 2), a region thought to contain the frontal eye fields. The left superior parietal cortex, posterior cingulate cortex, and occipital cortex also showed significant gray matter loss relative to controls. Subcortically, significant gray matter loss was identified, involving the caudate and putamen nuclei.

PSP Patients vs Controls

The most significant areas of atrophy observed in the 15 PSP patients relative to controls were in the midbrain and pons (Figure 1B, Table 4). In the midbrain, multiple voxels within a region that encompassed the medial longitudinal fasciculus and the nucleus of cranial nerve III, the central mesencephalic reticular formation, and the decussation of the superior cerebellar peduncles were significantly atrophied (Figure 1B, row 3). In the pons, a dorsal region, including the nucleus of cranial nerve VI, and portions of the parapontine reticular formation showed volume loss (Figure 1B, row 3; Table 4). The left caudate and right thalamus were also atrophied relative to controls. Bilaterally, there were small areas of gray matter loss (Figure 1B, row 2, red) in the frontal opercular cortex and white matter loss (Figure 1B, row 2, yellow) in the frontal, periventricular regions.

Table 1. Demographics for Patients and Controls*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>CBD Patients (n = 14)</th>
<th>PSP Patients (n = 15)</th>
<th>Control Subjects (n = 80)</th>
<th>ANOVA and t/Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/F, No.</td>
<td>4/10</td>
<td>9/6</td>
<td>37/43</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>64.6 (5.9)</td>
<td>70.9 (6.9)</td>
<td>67.9 (8.6)</td>
<td>F2,128 = 2.35</td>
</tr>
<tr>
<td>Education, y</td>
<td>14.8 (2.4)</td>
<td>16.3 (3.1)</td>
<td>17.7 (3.3)</td>
<td>F2,128 = 2.76</td>
</tr>
<tr>
<td>Disease duration, y</td>
<td>5.6 (1.7)</td>
<td>4.8 (1.7)</td>
<td>NA</td>
<td>F2,128 = 1.32</td>
</tr>
<tr>
<td>MMSE score</td>
<td>19.2 (8.3)†</td>
<td>24.0 (3.2)</td>
<td>NA</td>
<td>t = −2.025</td>
</tr>
</tbody>
</table>

Abbreviations: ANOVA, analysis of variance; CBD, corticobasal degeneration; MMSE, Mini-Mental State Examination; NA, not applicable; PSP, progressive supranuclear palsy.

†P = .05 vs PSP.

Table 2. Functional and Motor Ratings in Patient Groups*

<table>
<thead>
<tr>
<th></th>
<th>CBD Patients</th>
<th>PSP Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDR scale, scaled score</td>
<td>0.71 (0.58)</td>
<td>0.82 (0.58)</td>
</tr>
<tr>
<td>CDR scale, sum of boxes</td>
<td>3.88 (3.2)</td>
<td>5.39 (3.6)</td>
</tr>
<tr>
<td>Hoehn and Yahr scale score</td>
<td>3.0 (0.8)</td>
<td>3.3 (0.5)</td>
</tr>
<tr>
<td>Schwab and England scale score</td>
<td>52 (19)</td>
<td>48 (23)</td>
</tr>
</tbody>
</table>

Abbreviations: CBD, corticobasal degeneration; CDR, Clinical Dementia Rating; PSP, progressive supranuclear palsy.

*Data are mean (SD).

Table 3. Motor Findings and Symptoms at Time of Magnetic Resonance Imaging*

<table>
<thead>
<tr>
<th></th>
<th>No. (%) of CBD Patients (n = 13)</th>
<th>No. (%) of PSP Patients (n = 15)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow saccades</td>
<td>2 (15)</td>
<td>13 (87)†</td>
<td>.001</td>
</tr>
<tr>
<td>Vertical gaze limitation</td>
<td>3 (23)</td>
<td>15 (100)†</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Square wave jerks</td>
<td>0 (0)</td>
<td>9 (60)†</td>
<td>.001</td>
</tr>
<tr>
<td>Axial rigidity</td>
<td>4 (31)</td>
<td>15 (100)†</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Falls</td>
<td>5 (39)</td>
<td>15 (100)†</td>
<td>.001</td>
</tr>
<tr>
<td>Dystonia</td>
<td>9 (75)†</td>
<td>1 (7)</td>
<td>.001</td>
</tr>
<tr>
<td>Limb rigidity</td>
<td>13 (100)</td>
<td>11 (73)</td>
<td>.14</td>
</tr>
<tr>
<td>Limb apraxia</td>
<td>12 (92)†</td>
<td>2 (13)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Alien limb</td>
<td>2 (15)</td>
<td>0 (0)</td>
<td>.26</td>
</tr>
<tr>
<td>Cortical sensory deficit</td>
<td>9 (69)†</td>
<td>0 (0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Apraxia of speech</td>
<td>8 (61)†</td>
<td>2 (13)</td>
<td>.004</td>
</tr>
<tr>
<td>Myoclonus</td>
<td>4 (31)</td>
<td>0 (0)</td>
<td>.02</td>
</tr>
<tr>
<td>Dysarthria</td>
<td>4 (31)</td>
<td>5 (33)</td>
<td>.88</td>
</tr>
<tr>
<td>Tremor</td>
<td>3 (23)</td>
<td>0 (0)</td>
<td>.15</td>
</tr>
<tr>
<td>Levodopa benefit (subjective)</td>
<td>1 (8)</td>
<td>2 (13)</td>
<td>.63</td>
</tr>
</tbody>
</table>

Abbreviations: CBD, corticobasal degeneration; PSP, progressive supranuclear palsy.

*Thirty-four control subjects were also examined, and none were found to have any of these abnormalities.
†CBD greater than CBD, P < .05; χ² test.
‡CBD greater than PSP, P < .05; χ² test.
Direct Comparisons of Patient Groups

When the degree of gray and white matter loss was directly compared in the 2 patient groups, the CBDS group had more atrophy in the left frontal eye field and left superior parietal lobule than the PSP group (Table 4). In contrast, the PSP group displayed more atrophy than the CBDS group in the midbrain, in a region encompassing the central mesencephalic reticular formation, the nucleus of cranial nerve III, and the rostral portion of the medial longitudinal fasciculus (Table 4).

Patient Classification

To determine which regions of atrophy best differentiated the 2 patient groups, the gray or white matter concentration at each voxel listed in Table 4 was entered into a stepwise discriminant function analysis. The degree of atrophy in 3 brain regions, the midbrain tegmentum, the dorsal pons, and the left frontal eye field, differentiated the CBDS patients from the PSP patients with 93% accuracy (Figure 2). One patient in each clinically defined patient group was misclassified as belonging to the other patient group by the neuroimaging criteria. Standardized canonical discriminant function coefficients for the 3 brain regions were 0.578 (midbrain tegmentum), 0.517 (pontine tegmentum), and −0.815 (frontal eye field).

COMMENT

This is the first study to use voxel-based methods to directly compare brain atrophy patterns in CBDS and PSP patients and thus provides much greater neuroanatomical detail than previous brain imaging studies of these 2 disorders. This study demonstrates that the most significant differences in brain atrophy in CBDS and PSP patients with similar disease severity occur in regions involved in eye movement control. Consistent with our hypothesis, CBDS patients showed more atrophy of the left dorsal frontal and parietal structures than PSP patients in the vicinity of the frontal and parietal eye fields. Conversely, PSP patients displayed more atrophy of the midbrain structures, in the region of the oculomotor nucleus and central mesencephalic reticular formation, than CBDS patients. Compared with controls, both disorders were associated with atrophy of the basal ganglia structures; however, CBDS patients displayed prominent cortical volume loss, whereas PSP patients showed the most atrophy in the rostral brainstem. The degree of atrophy of these brain regions accurately differentiated patients with CBDS from those with PSP.

COMPARISON WITH PATHOLOGIC STUDIES OF CBD AND PSP

This study extends the findings of a recent autopsy comparison of brain atrophy between CBD and PSP, which demonstrated a greater degree of cortical atrophy in CBD than PSP,1 to show that the same differences in brain anatomy are appreciable during life at an intermediate stage of disease severity in patients with typical clinical presentations of the 2 disorders. The patterns of brain atrophy observed in our study are also highly consistent with the anatomical distribution of tau protein deposits in PSP and CBD. In CBD, we found that the most prominent region of atrophy was in the left superior frontal gyrus, which at autopsy usually contains the highest concentrations of ballooned neurons.2 In PSP, we found that the most prominent regions of brain atrophy were in the dorsal midbrain and pontine regions, which contain the ocular motor nuclei, and at autopsy consistently have high concentrations of neurofibrillary tangles.3 We were also able to document significant atrophy of the superior cerebellar peduncles (especially in the region of their decussation) in the PSP group, a neuroimaging finding suggested by a recent neuropathologic study.4

Figure 1. Regions of brain atrophy in patients with corticobasal degeneration syndrome (CBDS) and progressive supranuclear palsy (PSP) relative to controls. Voxel-based morphometry–identified regions of decreased gray and white matter volume relative to 80 age-matched control subjects in CBDS and PSP patients are displayed on a normal adult brain template (P<.05, corrected). A, CBDS patients vs controls. B, PSP patients vs controls. Row 1 shows the regions of significant gray matter loss rendered on a healthy subject’s brain. Row 2 shows regions of significant gray (displayed in red) and white (displayed in yellow) matter loss relative to controls at the following Montreal Neurological Institute (MNI) coordinates: x=−33, y=−4, and z=49. Row 3 shows regions of significant gray (displayed in red) and white (displayed in yellow) matter loss relative to controls at the following MNI coordinates: x=5, y=−15, and z=−8. A indicates anterior; P, posterior.
COMPARISON WITH PREVIOUS NEUROIMAGING STUDIES OF CBD AND PSP

The patterns of brain atrophy that were observed in our study of CBDs and PSP are consistent with previous MRI-based studies of brain atrophy in these disorders.1,5 Our data extend the results of a recent region-of-interest MRI analysis of pathologically confirmed cases of PSP and CBD.4 Consistent with this study, we demonstrated greater brainstem atrophy in PSP than in CBD and greater cortical atrophy in CBD than in PSP. The use of a voxel-by-voxel analysis of brain atrophy in our study allowed for much greater anatomical detail with similar accuracy in classification of patients based on fewer anatomical measurements. Specifically, we were able to identify regions within the frontal and parietal cortical regions where atrophy differentiates CBD from PSP. The regions identified are important for the cortical control of eye movement, making this imaging finding concordant with a major aspect of the clinical presentation in CBD. Although a similar pattern has been demonstrated in autopsy studies, this type of specificity has not previously been demonstrated in vivo. In contrast to the region-of-interest MRI analysis,4 which suggested a symmetric pattern of cortical atrophy in CBD, we identified an asymmetric pattern of cortical atrophy in the CBD group. This finding is nearly identical to the pattern of resting cerebral hypometabolism noted on statistical para-

<table>
<thead>
<tr>
<th>Anatomical Region</th>
<th>BA</th>
<th>x, y, z</th>
<th>z Score</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticobasal Degeneration (n = 14) vs Controls (n = 80)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left superior frontal gyrus (FEF)</td>
<td>6</td>
<td>−28, 3, 52</td>
<td>4.78</td>
<td>.02</td>
</tr>
<tr>
<td>Left superior frontal gyrus</td>
<td>6</td>
<td>−33, −4, 49</td>
<td>4.70</td>
<td>.02</td>
</tr>
<tr>
<td>Left superior parietal lobule</td>
<td>7</td>
<td>−18, −55, 67</td>
<td>4.71</td>
<td>.02</td>
</tr>
<tr>
<td>Left superior parietal lobule</td>
<td>7</td>
<td>−28, −47, 66</td>
<td>4.65</td>
<td>.03</td>
</tr>
<tr>
<td>Progressive Supranuclear Palsy (n = 15) vs Corticobasal Degeneration (n = 14)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midbrain tegmentum</td>
<td>NA</td>
<td>1, −18, −9</td>
<td>5.09</td>
<td>.003</td>
</tr>
</tbody>
</table>

Abbreviations: BA, Brodmann area; FEF, frontal eye field; NA, not applicable; WM, white matter.
*Montreal Neurological Institute coordinates.
†Multiple comparisons corrected, familywise error.

Figure 2. Magnitude of brain atrophy in regions that discriminate corticobasal degeneration syndrome (CBDs) from progressive supranuclear palsy (PSP). Brain volume (arbitrary units) from the voxel-based morphometry analysis is displayed for each patient at the 3 brain regions found to best discriminate the 2 patient groups. Dorsal midbrain volume is shown at the following Montreal Neurological Institute coordinates: x=5, y=−15, and z=−8. Dorsal pons coordinates are x=−1, y=−36, and z=−43. Left frontal cortex coordinates are x=−27, y=4, and z=54. Two patients were incorrectly classified by the discriminate function.

©2006 American Medical Association. All rights reserved.

Downloaded From:  on 10/05/2018
metric maps of positron emission tomograms with 18fluorodeoxyglucose of patients with CBD and subjective ratings of atrophy in CBD.3

CLINICAL-ANATOMICAL RELATIONSHIPS IN PSP AND CBD

From the earliest descriptions of PSP and CBD, it has been recognized that the control of eye movements is severely impaired by both disorders,20,23 and their distinctive saccade abnormalities can differentiate patients in each group.6

Our neuroimaging analysis demonstrated that the most significant areas of atrophy in both patient groups relative to controls were in brain regions involved in ocular motor control. In CBD, the intersection of the superior frontal sulcus and precentral sulcus, a region identified by functional MRI as the frontal eye field, was significantly atrophied relative to controls and PSP patients.19 In PSP, the dorsal midbrain and pons, regions involved in basic aspects of ocular motor control,7 were most atrophied. Thus, our results provide a potential neuroanatomical basis for saccade abnormalities associated with CBD and PSP. Further studies that correlate quantitative measures of saccades with regional brain atrophy are needed to test this hypothetical basis for CBD- and PSP-associated eye movement abnormalities.

Accepted for Publication: August 23, 2005.
Correspondence: Adam L. Boxer, MD, PhD, Memory and Aging Center, Department of Neurology, University of California, San Francisco, Box 1207, San Francisco, CA 94143-1207 (aboxer@memory.ucsf.edu).

Author Contributions: Study concept and design: Boxer, Geschwind, and Rosen. Acquisition of data: Boxer, Geschwind, Gorno-Tempini, Schauer, Miller, Weiner, and Rosen. Analysis and interpretation of data: Boxer, Geschwind, Belfor, and Gorno-Tempini. Drafting of the manuscript: Boxer, Geschwind, Belfor, Gorno-Tempini, and Schauer. Critical revision of the manuscript for important intellectual content: Boxer, Geschwind, Miller, Weiner, and Rosen. Statistical analysis: Boxer, Belfor, Gorno-Tempini, and Rosen. Obtained funding: Boxer, Miller, Weiner, and Rosen. Administrative, technical, and material support: Weiner. Study supervision: Boxer, Miller, and Rosen.

Funding/Support: This study was supported by grant K23 NS48855 (Dr Boxer) from the National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Md; the John Douglas French Foundation, Los Angeles, Calif; grant NIH AG19724 (Dr Miller) from the National Institutes of Health; a National Institutes of Health Alzheimer’s Disease Research Center grant; the Hillblom Foundation, Petaluma, Calif; the State of California; and the McBean Foundation, San Mateo, Calif.

REFERENCES