Neuroimaging studies are increasingly performed in macaque species, including the pig-tailed macaque (Macaca nemestrina). At times experimental questions can be answered by analysis of functional images in individual subjects and reference to a structural image in that subject. However, coregistration of functional brain images across many subjects offers the experimental advantage of enabling voxel-based analysis over multiple subjects and is therefore widely used in human studies. Voxel-based coregistration methods require a high-quality 3D template image. We created such templates, derived from T1-weighted MRI and blood-flow PET images from 12 nemestrina monkeys. We designed the macaque templates to be maximally compatible with the baboon template images described in a companion paper, to facilitate cross-species comparison of functional imaging data. Here we present data showing the reliability and validity of automatic image registration to the template. Alignment of selected internal fiducial points was accurate to within 1.9 mm overall (mean) even across species. The template images, along with copies aligned to the UCLA nemestrina brain atlas, are available on the Internet (purl.org/net/kbmd/n2k) and can be used as targets with any image registration software. © 2001 by Academic Press

INTRODUCTION

Functional neuroimaging studies have been conducted for some time in macaques, including in awake animals (Perlmutter et al., 1991). With the advent of fMRI, studies in awake macaques are receiving increased attention (Stefanacci et al., 1998; Logothetis et al., 1999). An important step is to extend single-subject reports to across-subject investigations that can address intersubject variability and generalizability (Woods, 1996; Friston et al., 1999a). One approach to this goal is to analyze functional responses by combining spatially normalized data across subjects (Friston et al., 1999b). Multisubject image averaging has only recently been applied to nonhuman species, and published methods in primates have various drawbacks, including untested quality (Tsujimoto et al., 1997; Chen et al., 1999) or labor-intensive procedures with dependence on human expertise (Black et al., 1997).

Several available methods provide reliable, unsupervised computer registration of brain images to an atlas template, but these methods require prior development of suitable 3D templates (Woods et al., 1998; Ashburner and Friston, 1999). For Macaca nemestrina (the pig-tailed macaque), one apparent candidate was the cross-modality atlas of Cannestra and colleagues at UCLA (1997). This dataset includes images from CT, MRI, and PET from a single animal, presented in register with a 3D reconstruction of anatomically labeled cryosection data from the same animal. We chose to create a new template averaged from brain MRIs from many different animals, for reasons elaborated in the Discussion.

In a companion report, we describe and validate our baboon template and demonstrate the accuracy of software that automatically registers individual MRI or PET images to these multisubject templates (Black et al., 2001). In this communication we describe multisubject template images for macaque corresponding to T1-weighted MRI and to PET [15O]water blood-flow images and show that the template-aligned MR images correspond closely to the baboon template.

METHODS

Subjects

The template images derive from 12 male, neurologically normal M. nemestrina monkeys. All images were acquired as part of studies approved by the Washington-
ton University Animal Studies Committee. Weights were available for 11 monkeys (min 7.2 kg, median 11.7 kg, max 13.5 kg). Ages were available for only 2 animals, who were 6.4 and 6.5 years of age and weighed 11.25 and 10.2 kg, respectively.

**Image Acquisition**

Three-dimensional sagittal MPRAGE images and PET blood-flow images were acquired as described (Black et al., 2001). The MRI template derives from 1 MPRAGE image from each monkey, while the PET template comes from 104 H\textsubscript{2}O\textsubscript{15} images obtained in 9 of the 12 monkeys.

**Image Registration**

We registered images using custom computer programs described elsewhere (Black et al., 2001); for registration of cryosection data to MRI we used the same cross-modality registration method as for PET to MR images.

**Template Image Development**

Template images were created iteratively as previously described (Black et al., 2001), except that at each step the composite macaque MRI image was realigned to the “b2k” baboon MRI template to ensure optimal cross-species compatibility. We then aligned a copy of the macaque MRI template to the UCLA cryosection atlas. To avoid accumulation of interpolation errors, we created the UCLA-compatible template by multiplying the matrices that represent the subject-to-MRI-template and the MRI-template-to-cryosection transforms and then resampling the original MRI data in a single step. The method used to create the PET template in register with the MRI template was described and validated in the companion paper (Black et al., 2001).

**Validation**

We tested the within-species accuracy of the matching-to-template process as follows. We first spatially normalized the original MPRAGE from each subject to match the macaque MRI template image. In each of these 12 normalized images, one author identified the location of eight test points. For each test point in each macaque, we then computed error (3D distance) compared to the mean location for that point across all 12 animals. We also tested the accuracy of matching across species by comparing these same test points to the expected locations for these points from the baboon template (Black et al., 1997, 2001).

**RESULTS**

**Template Images**

Using the methods above, we formed an MPRAGE template image, “n2k,” and a corresponding PET blood-flow template image, “n2kf.” The MRI template, although a composite of 12 scans, shows clear demarcation of many cortical and subcortical structures (Fig. 1). Both template images are available on the internet (Black et al., 2000). Copies of the MRI and PET templates, aligned to the UCLA atlas, is available at the same location (Fig. 2).

**Validation**

We quantitatively tested the subject-to-MRI-template process for each of the 12 macaque brain images...
used to make the template. Test points fell within an average of 1.2 mm of the sample mean location in the 12 images (maximum, 3.4 mm; see Table 1). Compared across species to the expected locations from the baboon template image, accuracy was still reasonable, with a mean error of 1.9 mm (maximum, 4.9 mm; see Table 1).

**DISCUSSION**

We present multisubject, T1-weighted MRI and PET blood-flow template images of *M. nemestrina* brain for use as targets of automatic, voxel-based image registration methods. Additionally, we demonstrate that alignment of individual macaque brain MR images to the macaque MRI template provides a reasonably accurate correspondence of selected brain landmarks to the same landmarks in the baboon MRI template.

The measured cross-species accuracy (1.9 mm mean error) subsumes all of the following: human error in identification of brain landmarks, image registration error, true nonlinear morphologic differences among the individual macaque brains, morphologic differences between species, and the degree to which the baboon atlas (from which we derived the expected locations for subcortical points) is atypical of living baboon brains. The reasonable between-species fit is not entirely surprising, since we had previously shown that a macaque brain image was linearly aligned to a baboon atlas as accurately as several baboon brain images (Black *et al.*, 1997). The macaque template brain image, matched as it is to a baboon template, is larger than the original macaque brain images (Table 2). However, the alignment software accounts for this, and some individual macaque brains were similar in size to the template. The templates were made from images of male monkeys. However, female and male baboon brain images were registered to the template with similar accuracy (Black *et al.*, 2001, and unpublished observations).

These new template images address several unmet needs for neuroimaging research in nonhuman primates. Older works such as the Winters *et al.* (1969) printed atlas do not include adequate 3D data for automated image registration. Furthermore, that atlas was based entirely on animals weighing 3 to 4 kg and assumes uniformity of brain size. In larger animals,
TABLE 2
Average Stretch Values Computed from the Transformation Matrix Obtained by Registering Each of 11 Macaque MPRAGE Images to the n2k Template

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>x stretch (left-right)</td>
<td>0.906</td>
<td>0.831–0.980</td>
</tr>
<tr>
<td>y stretch (anterior–posterior)</td>
<td>0.830</td>
<td>0.661–0.991</td>
</tr>
<tr>
<td>z stretch (superior–inferior)</td>
<td>0.838</td>
<td>0.712–1.022</td>
</tr>
</tbody>
</table>

Note: On average, the 11 original individual brains were roughly 9% narrower (x), 17% shorter (y), and 16% shorter (z) than the n2k template brain, reflecting the intent of the n2k template to correspond as closely as possible to the b2k baboon template (Black et al., 2001). However, brain size in individual macaques overlapped the brain size of the individual baboons that contributed to the b2k baboon template.

our experience is that labels from that atlas no longer correspond well to individual brain images. Similar obstacles prevented our use of atlases available for other macaque species (e.g., Martin and Bowden, 1996; François et al., 1996; Paxinos et al., 2000).

The UCLA atlas of M. nemestrina includes labeled cryosections matched to CT, PET, and MR images from a single animal. We chose to create a new template for several reasons. First, MR images of much higher quality are available now than at the time the UCLA atlas was constructed, and we would expect better consistency of registration to images of similar technique. Second, we wanted the macaque template to correspond as closely as possible to our baboon template, since we compare responses between the two species (Black et al., 2001; Hershey et al., 2000). And third, we wanted a template image averaged from a number of subjects, to better represent population variability in anatomy. Single-subject functional imaging studies often can be interpreted by reference to a structural image from the same subject. However, when one desires to interpret combined functional imaging data from a population sample, it is advantageous to reference responses to a structural template that represents an "average" image (Black et al., 2001). Observations similar to these have led to the common use of multi-subject target images for human imaging, for instance the templates supplied in the standard distribution of SPM99 software (Mazziotta et al., 1995). We provide copies of the new macaque templates in register with the UCLA atlas for investigators who want to refer functional imaging results to that source.

We believe the availability of these template images will facilitate analysis of neuroimaging studies in macaque. Only recently have investigators interpreted functional imaging in any nonhuman species by combining data across different animals, and methods have been awkward or have had unknown accuracy (reviewed in Black et al., 2001). (Very recently Cross et al. (2000) reported an independent approach to the same problem.) The method we now present is superior to the older methods both in practical terms and in terms of reliability and accuracy.

ACKNOWLEDGMENTS

We thank Terry Anderson, Ryan Collins, and Bettina Tobben for technical assistance and Drs. Tamara Hershey and Jonathan Mink for some of the images used. Supported by NIH Grants NS01898 and NS31001, the Tourette Syndrome Association, the Greater St. Louis chapter of the American Parkinson Disease Association, the Mary and Robert Bronstein Foundation, the McDonnell Center for the Study of Higher Brain Function, and a Young Investigator Award to K.J.B. from NARSAD (the National Alliance for Research on Schizophrenia and Depression).

REFERENCES


