

# Anatomic Standardization, Although Controversial, Finds Yet Another Application

Anatomic standardization appears to be a universal trend. It was originally used to localize activation foci in PET activation studies. Then it was used to find hypometabolic areas in FDG PET images. Although the technique is still controversial for the human brain, especially regarding use for patients, in this issue of *The Journal of Nuclear Medicine* Cross et al. (1) have applied a standardization technique called 3-dimensional stereotactic surface projections (SSP) to images of the cerebral metabolic rate for glucose (CMRglc) of the monkey brain.

## ANATOMIC STANDARDIZATION VERSUS REGISTRATION

Let me first clarify the difference between anatomic standardization (also called spatial normalization) and registration in the narrow sense of the word (Table 1). Anatomic standardization is a technique to transform the brain images of each subject into a standard brain, whereas registration matches an image to another, usually of a different modality or tracer, of the same subject through a rigid-body transformation (i.e., 3-dimensional shift and rotation). Some people may misunderstand the techniques as similar processes. This is partly because the term "registration" is also used in a broad sense as intersubject registration, to represent anatomic standardization, and partly because the same computer algorithm can be used for both processes and, therefore, many software packages support both. In fact, both processes transform an image to match another so that a cost

function is minimized, and an algorithmic difference lies in whether a nonlinear or rigid-body transformation is used. Even a non-rigid-body transformation may be used for PET-MRI registration to account for distortion in MRI. Another source of confusion is the use of registration as part of some anatomic standardization processes. However, one should understand clearly that these 2 processes are conceptually different.

Registration is an analysis of an individual subject. The purpose is to examine the subject from various aspects, such as morphology (using MRI and CT), blood flow, and metabolism. For example, registration is used to examine the topographic relationship between the gyri, a mass lesion, a viable tumor, and the eloquent cortex revealed as activation foci (2). On the other hand, anatomic standardization analyzes subject groups, for which individual variations are treated statistically. In fact, statistical parametric mapping (SPM) (3), which is the most popular method of standardization, is incorporated in a package together with software for statistical analysis. SPM has been used to reveal which parts of the brain defined in the atlas are significantly activated by a task for a group of subjects. Recently, SPM was also used to find out which parts of the brain have a significant difference in cerebral blood flow (CBF) between healthy volunteers and patients with Parkinson's disease (4). One may argue that the examination of a patient image in the standardized coordinate system to detect pixels above or below the normal range appears to be an individual analysis. However, this is really an analysis of a group, because the patient is compared with a group of healthy volunteers and the result depends on the selection of the volunteers.

Another important difference is that the true solution exists for registration but not for anatomic standardization. For a pixel in a brain image, in principle there is always a corresponding point (pixel) within every other brain image of the same subject, although finding it or demonstrating that it is the point may not be easy. However, no single true solution exists for anatomic standardization. No universal criteria exist for determining that a certain point in the brain of a subject corresponds to a certain point in another subject. Every brain is different from another both morphologically and functionally. The gyral pattern is known to be topologically different between subjects (5). Functional differentiation is also different between subjects, even when it is tracked down to the neural network at the microscopic level. Otherwise, everybody would think and behave in exactly the same way as everybody else. Therefore, no universal criteria can determine that a method of anatomic standardization is superior to another. Determining which method is best depends on the purpose of the study. A variety of methods are available for anatomic standardization, and SPM has several versions and options.

Many investigators refuse to apply anatomic standardization to patients because every patient is unique, even if he or she has the same disease as another patient. Meanwhile, many other investigators are willing to apply standardization. Some argue that morphologically normal patients can be standardized. Using the utmost caution when standardizing patients is reasonable. However, rejecting standardization totally is theoretic extremism. In fact, region-of-interest analysis, which has been performed for decades, is

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**TABLE 1**  
Conceptual Difference Between Anatomic Standardization and Registration

Parameter	Anatomic standardization*	Registration†
Materials	Images of different subjects Same modality or tracer	Images of same subject Same or different modality or tracer
Transformation	Nonlinear in general	Rigid body
Standard brain	To be used	Not to be used
Atlas	Applicable and available	Not essential, only used as a reference
Solution	No single true solution	Single true solution exists
Study subject	Population	Individual
Analysis	Statistical analysis	Multilateral examination

\*Also called spatial normalization.  
†In the narrow sense.

regarded as a sort of manual standardization with strong smoothing.

### LANDMARKS VERSUS ACTIVITY DISTRIBUTION

Standardization methods are classified into 2 major approaches. In the first approach, the brain of each subject is transformed so that the landmarks match those in the standard brain, whereas in the second approach it is the radioactivity distribution (or CBF or CMRglc) that one attempts to match. The former includes the human brain atlas (6), which uses morphologic information provided by PET-registered MRI. The latter includes SPM of various versions and options (3) and the so-called Michigan method developed by Minoshima et al. (7). It is noted that this classification represents 2 approaches to the goal, and the Michigan method also adopts a landmark matching process for the bicommissural line.

This classification reflects the inherent difference in the philosophy of standardization. When landmarks such as contour and sulci are matched, morphologically corresponding pixels from each subject are compared and averaged in a way that is a natural extension of drawing regions of interest by visual inspection. Accordingly, the standardization may be somewhat valid for patients with morphologic derangement if the landmarks are accurately extracted and matched. When PET activation data are standardized in this manner, however, morphology is assumed to govern the functional differ-

entiation: the precentral sulcus of 1 subject functionally corresponds to the precentral sulcus of another subject. This correspondence is not always true. Furthermore, intersubject variation in CBF images standardized with the human brain atlas was surprisingly large at the edge of gray matter, probably because of an inconsistent relationship between landmarks and CBF distribution as well as an error in PET-MRI registration (8).

When the activity distribution is to be matched to the template, activity is assumed to represent anatomy. Minimization of intersubject variations in activity in subsequent statistical analyses is the objective and is beneficial for PET activation analyses (8). Because no landmark information is used, morphologic matching is not guaranteed. Therefore, application to patients requires extra caution because incorrect distortion may occur for subjects with an abnormal activity distribution even if normal morphology is maintained. Interestingly, the major sulci of all but a few healthy volunteers were mapped to similar positions by version SPM95 of SPM, which did not use any landmark information (9).

### RESPONSE TO STIMULI VERSUS RESTING VALUE

Anatomic standardization was initially used to detect significant activation foci in PET activation studies, in which the statistical significance of the difference in CBF (or radioactivity) between 2 conditions was tested. In

other words, the response of CBF to a stimulus was being evaluated (type A). Anatomic standardization is now also used to compare the distribution of CBF (or radioactivity) itself between groups or to examine its correlation with external variables (e.g., age) (type B). These 2 types of statistical analysis (summarized in the Appendix) are essentially different in handling the intersubject mismatch in CBF. In type A, the individual variation in CBF is removed as the subject effect, whereas in type B, the individual difference in (resting) CBF is the target of analysis.

The greatest caution should be used when the activity distribution is compared between subject groups (type B designs) if the images have been standardized by matching the activity distribution (i.e., with SPM or Michigan), because intersubject differences would disappear under complete standardization. In other words, if an area is found to have decreased activity in a standardized subject image, pathologic hypoactivity cannot be differentiated from incomplete standardization. This is an essential contradiction of using a single set of information both for anatomic standardization and for statistical comparison.

### VOLUME IMAGE VERSUS SURFACE PROJECTION

Minoshima et al. (10) developed a method of projecting the cortical activity (or CBF or CMRglc) visualized in a 3-dimensional volume image onto the brain surface to create a surface repre-

sensation of the cortical activity distribution. This method has been combined with the previously developed Michigan standardization method, and the entire process is named 3-dimensional SSP (10). Minoshima et al. (11) applied it to FDG PET analysis to find hypometabolic areas in patients with early Alzheimer's disease.

Anatomic standardization by 3-dimensional SSP is a combination of volume image standardization and surface projection. The effects of both parts should be considered when any results regarding the method are discussed. Investigators who compare 3-dimensional SSP with SPM, for example, should remember that SPM does not have the second part of 3-dimensional SSP.

Practically, surface projection has the advantage of erasing the radial mismatch between subjects in radioactivity distribution, which remains after standardization on volume images. Because of the partial-volume effect, the activity distribution within the cortical rim of healthy volunteers reflects the distribution of gray matter more than that of radioactivity per milliliter of gray matter. Therefore, surface projection erases the mismatch in gray matter distribution in the radial direction. Surface projection is also beneficial in reducing data size and forming an explanatory display for nonprofessionals.

The surface projection loses information about radial profiles within the cortical rim and deep structures. Surface projection is based on the notion that the cerebral cortex is essentially a 2-dimensional sheet of laminar structure bent and folded in 3-dimensional space and that, therefore, the radial profile information is trivial. This notion may be true for gyral areas but not for sulcal areas. On an area covering a sulcus, the algorithm of 3-dimensional SSP projects the maximum activity inside the sulcus. To strictly realize the notion of a 2-dimensional sheet, sulci must be unfolded or flattened, requiring far higher resolution than PET images provide, and being applicable only to MRI (12).

Surface projection causes substantial loss of spatial resolution from the original volume image. This issue is not important as far as the cortex is concerned. When a set of volume images is analyzed as it is (e.g., with SPM), strong smoothing is always performed before statistical analysis to reduce intersubject mismatch, making the resolution far lower than that of the original images. Loss of spatial information, being heavier in sulcal areas than in gyral areas, is uneven in 3-dimensional SSP. However, uneven smoothing occurs in the course of nonlinear standardization on volume images, whether SPM or 3-dimensional SSP.

#### APPLICATION TO MONKEY BRAIN

Nonhuman primates have been a target of neuroscience research because the organization of their brains is closer, both morphologically and functionally, to that of the human brain than is that of the brains of other animals. Of the primates, macaques are the most extensively studied, using techniques that include histochemical staining, neuroanatomic tracing, single-cell recording, optical imaging, and autoradiography. These investigations are often combined with behavioral studies of awake animals. Accordingly, PET measurement of the regional distribution of radioactivity (or CBF or CMRglc) and its age-related changes has become a matter of interest (13), as has localization of task-related activation foci using the PET-activation technique (14). To facilitate identification of anatomic areas on the images, a stereotactic atlas has been created (15). Cross et al. (1) have created a standard template of the macaque brain and transformed each subject's image onto the template, thereby exploring the possibility of pixelwise statistical analysis by intersubject averaging. As a result, they have found age-related changes in regional CMRglc. Because the macaque brain is of a different shape from the human brain, they modified the 3-dimensional SSP software.

Application of anatomic standardization to animal brains has just begun, and its validity has been neither estab-

lished nor tested by many investigators. Because anatomic standardization is already controversial for human brains, especially for patients as discussed above, its use on animals will create another matter for controversy. Still unknown is the extent to which the pixelwise statistical significance obtained with this type of study can contribute to neuroscience research on monkeys. Because anatomic standardization is subject to error by morphologic derangement, application will be limited to healthy animals or to those in the early degenerative state. This limitation will be a drawback, because the availability of many animal disease models is a major reason for the raising and use of animals for experiments.

Another potential use for anatomic standardization of animal brains lies in the field of veterinary medicine. Just as the standardized brain image of an individual human patient is compared with the normal range pixel by pixel, the standardized image of an animal patient may be compared with a normal group for automated diagnosis. Application of nuclear medicine to veterinary medicine for the diagnosis and treatment of diseases in house pets, racehorses, endangered species, clones, and other laboratory animals is a promising field, and so is veterinary PET (16). In aged dogs, the presentation of signs similar to human dementia is a matter of significance for the owners as well as for neuroscience researchers (17). Although limited to animals with minimal morphologic derangement, anatomic standardization may, if validated for each species and subspecies, be useful for the clinical or preclinical diagnosis of brain disorders in such animals.

#### CONCLUSION

Anatomic standardization is coupled to statistical analysis on groups and is a concept totally different from registration. No single method is best, and the choice of method depends on the purpose of the study. In general, application to patients should proceed with the utmost caution. Standardization methods are classified into 2 approaches:

matching landmarks or matching activity distribution. The latter requires caution if the activity itself is to be compared between subject groups. The surface projection technique reduces intersubject mismatch and may be appropriate for comparing activity distribution in the cortex. Surface projection has recently been applied to the macaque brain but is yet of undetermined usefulness.

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## APPENDIX

### Two Statistical Designs for Intersubject Averaging Analysis on Standardized Images

Type A designs contain a subject effect, and the statistical significance of another effect is tested.

A1. Acquire CBF (Y) images repeatedly under different conditions (j) (e.g., rest and task) for each subject (i) and determine whether a significant difference in CBF exists between the conditions (i.e., discover a task-induced increase):

$$Y_{ij} = \mu + \alpha_i + \beta_j + \epsilon, H_0: \beta = 0.$$

A2. Classify subjects into groups (k(i)), acquire CBF (Y) images repeatedly under different conditions (j) (e.g., rest and task) for each subject (i), and determine whether a significant difference in task-induced CBF increase exists between the groups:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \gamma_{jk} + \epsilon, H_0: \gamma = 0.$$

A3. Using subjects of various ages (x(i)), acquire CBF (Y) images repeat-

edly under different conditions (j) (e.g., rest and task) for each subject (i), and determine whether a significant correlation exists between age and task-induced CBF increase:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \gamma_j x + \epsilon, H_0: \gamma = 0.$$

Type B designs do not contain a subject effect. The statistical significance of the main effect of a factor, within which the subject is nested, is tested.

B1. Classify subjects into groups (k(i)), acquire a CBF (Y) image for each subject (i), and determine whether a significant difference in CBF exists between the groups:

$$Y_i = \mu + \delta_k + \epsilon, H_0: \delta = 0.$$

B2. Using subjects of various ages (x(i)), acquire a CBF (Y) image for each subject (i), and determine whether a significant correlation exists between age and CBF:

$$Y_i = \mu + \delta x + \epsilon, H_0: \delta = 0.$$

Note. CBF may be replaced by radioactivity or CMRglc.

$\epsilon$ : error term.  $H_0$ : null hypothesis.

Regional values are assumed to be normalized by the global or reference tissue value.

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