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Impact of inter-subject image registration on group analysis of fMRI data

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Abstract. A comparative study of three inter-subject brain image registration methods (SPM99, AFNI, and ART) is presented. It is shown that ART, which has a greater degree of freedom than SPM99 or AFNI, is able to more accurately remove the anatomical variability between high-resolution MR images of different subjects. The accuracy is assessed by the ability of the algorithm to reduce a measure of spatial dispersion among manually selected, homologous landmarks. We also investigated whether the superior ability of ART in removing inter-subject anatomical variance has any advantages for group analysis of functional magnetic resonance imaging (fMRI) data. In this study, data from a group of 21 subjects performing the visual oddball task were analyzed using three registration methods. The impact of inter-subject registration on the resulting activation maps was assessed using reproducibility and sensitivity measures derived from a nonparametric statistical analysis of the data. Using these measures, it is shown that a statistically significant increase in the reproducibility of activation maps and empirical sensitivity of activation detection can be achieved when ART is used for inter-subject registration. We conclude that there are significant advantages to be gained by using high dimensional, inter-subject registration methods for group analysis of fMRI data. © 2004 Elsevier B.V. All rights reserved.

Keywords: Brain; Image registration; Spatial normalization; Functional MRI; Visual oddball task

1. Introduction

A necessary step for group analysis of functional magnetic resonance imaging (fMRI) data is *inter-subject image registration* or *spatial normalization* [1]. The aim of inter-subject brain image registration is to establish a one-to-one continuous correspondence or *homeomorphism* between the brains of two different individuals. The transformation will reduce the anatomical variability between brain images from different subjects. This

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enables the analyst to compute a single activation map representing the entire group of subjects or to compare the brain activation between two different groups of subjects (e.g., patients vs. normal controls).

Several different approaches have been proposed for solving the inter-subject brain image registration problem in MRI. These approaches include piecewise linear transformations with relatively few parameters [2,3], nonlinear deformations with several hundred degrees of freedom [4,5], and highly flexible deformations with thousands of degrees of freedom [6–10]. Thus, the fMRI data analyst is faced with the question of whether there are any advantages in using one method of registration over another. The aim of this chapter is twofold: (1) investigate how different inter-subject registration algorithms compare in terms of registration accuracy, and (2) assess the impact of increased registration accuracy on group analysis of fMRI data.

We conducted a quantitative comparison of registration accuracy between three programs: AFNI [3], SPM99 [4], and ART [10]. These are representatives of low, medium, and high dimensional, inter-subject registration algorithms. Each method was used to register high-resolution, T_1-weighted MRI volumes within the same group of subjects. Validation of registration accuracy was based on the ability of the algorithms to register manually identified, homologous landmarks. The hypothesis in this experiment was that the high dimensional, inter-subject registration algorithm implemented in ART is able to reduce the anatomical variability between subjects to a greater extent as compared with low/medium dimensional transformations produced by AFNI and SPM99. However, a reduction in anatomical variability may not necessarily result in greater sensitivity/specificity in the group analysis of functional images. This is due to the fact that there may be significant functional variability between subjects that would overshadow any differences in anatomical registration accuracy.

In order to investigate this question, that is the impact of different inter-subject registration algorithms on group analysis of fMRI data, we collected fMRI and high-resolution anatomical images from a group of subjects performing a *visual oddball* task. This task involves higher cognitive functions and is known to activate a wide network of brain regions [11–13]. We performed three different group analyses on these data. All the steps in these analyses (motion-correction, feature extraction, interpolation, etc.) were exactly the same except for the inter-subject registration procedure, where in each case a different algorithm (AFNI, SPM99, or ART) was applied. The impact of inter-subject registration was assessed in terms of reproducibility and sensitivity using a nonparametric *resampling* approach similar to that proposed in the NPAIRS data analysis framework [14].

2. Materials and methods

2.1. fMRI stimulation paradigm

The subjects in our experiments were given the "classic visual oddball task" during fMRI data collection [11]. In this paradigm, a series of visual stimuli were presented to the subject at regular intervals (1648 ms). The visual stimuli were either the *standard* type, which occurred more frequently (93.75% of times), or the *target* type. Both the standard and the target stimuli consisted of a string of white characters on a dark background (Fig. 1).

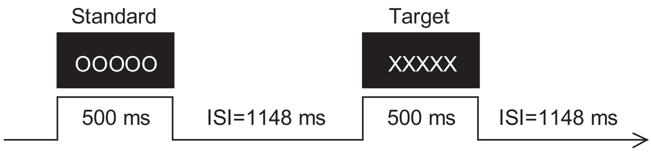


Fig. 1. The standard and target stimuli and trial design used in the f MRI study.

The characters were "OOOOO" for standard events and " XXXXX" for target events. The subject was instructed to silently count the number of target events and report the total number at the end of the experiment. The stimuli were delivered to the subject via a liquid crystal display (LCD) mounted on the MRI scanner's radio frequency (RF) head coil. The LCD display was connected to the video graphics array (VGA) output of a personal computer (PC) outside the scanner room, running the E-PRIME stimulus presentation software (Psychology Software Tools, Pittsburgh, PA, USA). A total of 1024 images were shown to the subject (64 targets and 960 standards). Stimulus duration was approximately 500 ms and the interstimulus interval (ISI) was 1148 ms, during which time the screen was dark. The 64 target events were distributed randomly among the 1024 trials, but the distribution pattern was identical for all subjects in the study.

2.2. MR imaging

The 21 subjects in this study were volunteers with no history of major medical or psychiatric illness (5 females and 16 males, ages 21–49, mean $\pm$ S.D. age = 27 $\pm$ 6 years). The local Institutional Review Board (IRB) approved all procedures. Written informed consent was obtained from all participants.

The MRI scans were performed using a 1.5 T Siemens VISION system (Siemens, Erlangen, Germany) with a quadrature birdcage head coil for both RF transmission and reception. The subjects wore earplugs and lay supine on the scanner bed with cushions placed around their heads to reduce motion.

Functional imaging consisted of collecting 1024 volumes in four runs of 256 using a T_2*-weighted, gradient echo, single-shot, blipped, echo-planar imaging (EPI) sequence (TR = 1648 ms, TE = 45 ms, $\alpha = 90°$, FOV = 250×250 mm^2). There was a slight delay (less than 10 s) between experimental runs, which was required to reload the MRI scanner pulse sequence. Each EPI volume had 15 axial slices measuring 64×64 pixels with 6 mm thickness and no gap. The slices were acquired approximately parallel to the anterior to posterior commissural line (AC-PC line). The scanner was triggered by a TTL pulse generated by the E-PRIME stimulus presentation software to synchronize each volume acquisition with the onset of a visual stimulus. Due to the limited axial coverage of the EPI scans, the anterior inferior temporal lobe region and the inferior cerebellum were not imaged.

In addition to the functional data, a high-resolution, 3-D, T_1-weighted volume was acquired from each subject using a magnetization-prepared rapid acquisition gradient echo (MPRAGE) sequence (TR = 11.6 ms, TE = 4.9 ms, $\alpha = 8°$, FOV = $256 \times 256 \times 190$ mm^3, 1 mm^3 isotropic voxel size).

2.3. Motion correction

All image processing and analyses were performed on a 2.4 GHz Dell Precision Workstation 530 computer (Dell Computer, Austin, TX) running the SUSE Linux 7.3 operating system (SUSE, Oakland, CA, USA). The first four scans from each of the four experimental runs of 256 were discarded to ensure steady state magnetization, leaving a total of 1008 EPI volumes to be analyzed for each subject. Motion detection and correction were performed on the remaining 1008 volumes using the motion correction algorithm of the AFNI software package [3]. Accuracy of this motion correction method was validated in a separate study [15].

2.4. Intra-subject registration

The MPRAGE volume of each subject was registered to the average motion-corrected EPI volume using a rigid body registration specified in terms of three translation and three rotation parameters. These parameters were initially directly calculated from the positioning information available in the Siemens VISION file header. This information gives the exact position and orientation of each image slice and, therefore, each image voxel with respect to the magnet coordinates system. The six parameters were used to construct a 4×4 rigid body transformation matrix operating in the homogeneous coordinate system. This directly calculated transformation matrix was pre-multiplied by a second computed rigid body transformation matrix that accounted for possible small subject motion that may have occurred between EPI and MPRAGE acquisitions. The latter matrix was obtained using an optimization procedure that maximized the *mutual information* between the average motion-corrected EPI and the MPRAGE volume. The resultant 4×4 matrix was used to register the MPRAGE volume of each subject to their motion-corrected EPI volumes.

2.5. Inter-subject registration

The 21 intra-subject, registered MPRAGE volumes were cropped to remove the extracranial regions. This was accomplished in two steps: (1) using the Brain Extraction Tool (BET) of the FSL software package (Oxford Centre for Functional Magnetic Resonance Imaging, Oxford University, UK), and (2) using the MEDx software package (Sensor Systems, Sterling, VA, USA) to manually remove the extracranial regions that still remained after the first step.

Following this step, all 21 volumes were registered into the Talairach space [2] using AFNI. In addition, all volumes were registered to a *target* volume in Talairach space using SPM99 and ART. The target volume was selected to be the subject with the median brain volume (including CSF). Thus, for each of the 21 subjects, three registered MPRAGE images were obtained using AFNI, SPM99, and ART. In SPM99, the user has the option of selecting the number of nonlinear basis functions and the level of regularization. These parameters were left at their default values of $7 \times 8 \times 7$ with “medium” regularization.

2.6. Assessment of inter-subject registration accuracy

The accuracy of inter-subject registrations was assessed qualitatively by inspecting the average of the 21 registered images resulting from the application of each of the three algorithms. The more accurate registration method was expected to result in a sharper average. For a quantitative comparison, 20 landmarks on each of the 63 registered volumes (21 subjects × 3 methods) were manually located by one of the authors (AB). These landmarks consisted of the right and left frontal poles, right and left temporal poles, superior aspect of the anterior commissure (AC), superior aspect of the posterior commissure (PC), most anterior point of the corpus callosum (CC), most posterior point of CC, right and left frontal horns, right and left occipital poles, right and left anterior and posterior aspects of the putamen, right and left anterior aspect of the caudate nucleus, and right and left inferior aspect of the lateral extension of the fourth ventricle. Landmarks were located using in-house software written in IDL (Research Systems, Boulder, CO). The operator who selected the landmarks was blind to the registration method used in each case. That is, he did not know which registration algorithm (AFNI, SPM99, or ART) had been used to register each of the 63 volumes. The volumes were examined in random order. Thus, we obtained the coordinates of 20 different landmarks in each of the 63 volumes. Following landmark selection, the 63 coordinates of each landmark were divided into three sets of 21, corresponding to the three registration methods used. The dispersion of each set of 21 homologous landmarks within a registration method was calculated by the following formula:

$$d = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\|\boldsymbol{p}_i - \bar{\boldsymbol{p}}\|^2} \tag{1}$$

where N is the number of subjects, $\boldsymbol{p}_i$ are the position vectors of landmarks, and $\bar{\boldsymbol{p}}$ is their average. This resulted in a 20 × 3 (landmarks × methods) table of landmark dispersion measures, which was subsequently analyzed using a variety of statistical methods.

2.7. Statistical analysis of fMRI

A hemodynamic response function (HRF) in the form of a gamma function was assumed as follows:

$$h(t) = \begin{cases} e^{-t/\sqrt{\delta\tau}}\left(\dfrac{et}{\tau}\right)^{\sqrt{\tau/\delta}} & t > 0 \\ 0 & t < 0 \end{cases} \tag{2}$$

where the parameter $\tau = 4.7$ s marks the peak of the impulse response function, and $\delta = 0.06$ is a dimensionless parameter that controls its shape. Assuming that the times of occurrence of the $T = 64$ target events were $\{t_1, t_2, \ldots, t_T\}$, the expected BOLD response at activated voxels was modeled by:

$$\boldsymbol{r}(t) = \sum_{i=1}^{T} h(t - t_i) \tag{3}$$

This continuous function of time was sampled every TR = 1.648 s to yield a reference vector of length 1008. This reference vector was normalized to have a magnitude of 1, denoted by $\boldsymbol{r}$.

Following motion correction, we obtained a time series of length 1008 at each voxel in each subject. Each time series was centered so as to have a mean of zero. In addition, a linear component was subtracted from each time series. Mathematically, the resulting time series became orthogonal to the constant vectors $[1\ 1\ 1\ \ldots\ 1]^T$ and the linear vector $[1\ 2\ 3\ \ldots\ 1008]^T$. We let $\boldsymbol{s}_i$ represent the resulting corrected fMRI time-series at voxel i. A within subject, BOLD contrast, statistical parametric map (SPM-c) was calculated by simply taking the dot product of $\boldsymbol{s}_i$ and $\boldsymbol{r}$ at each voxel. Since the reference vector is normalized, this gives the magnitude of the component of $\boldsymbol{s}_i$ that is parallel to $\boldsymbol{r}$. This measure, denoted by c_i, was taken to reflect the strength of the activation at voxel i within the subject. To summarize, for each subject an SPM-c image volume was computed where each voxel i had a gray level c_i.

Recall that for each subject, we obtained three nonlinear transformations representing inter-subject registrations, using AFNI, SPM99, and ART. These three transformations were applied to the SPM-c of each subject, thus obtaining three sets of spatially normalized SPM-cs for each subject. Finally, to obtain activation maps for a group of subjects, a one-sample t-statistic was computed at each voxel of the spatially normalized SPM-cs for testing the null hypothesis that the average BOLD contrast in the subject population is zero at the voxel under consideration. The resulting maps were denoted as SPM-ts. For any group of subjects, the methodology described yields about three SPM-ts. All the processing for obtaining these maps is exactly the same, except for the inter-subject registration method where for each case, AFNI, SPM99, or ART is used. Evaluation of these maps allows quantitative comparison of the impact of inter-subject registration on group analysis of fMRI data.

2.8. Reproducibility of fMRI activation

There are 352,716 possible different ways of dividing 21 subjects into a group of 10 and a group of 11 individuals. From these possibilities, we randomly selected 100 groupings and performed two analyses on the resulting groups of 10 and 11 subjects, using the procedure described above. Therefore, we obtained a pair of SPM-ts for each of the 100 random group selections. The two maps were thresholded at $p = 0.01$ (two-tailed). This classified each voxel as one of three types: significantly positively activated, significantly negatively activated, and nonactivated. Let N_1 and N_2 be the number of activated pixels (positive or negative) in groups 1 and 2, respectively, and A be the number of voxels where both groups show the same sign activations (i.e., they are in agreement), and D be the number of voxels in which one group shows a positive activation while the other group shows a negative activation (i.e., they are in disagreement). We define the *activation reproducibility measure* (ARM) as the coefficient $\rho = 2(A - D)/(N_1 + N_2)$ as a measure of reproducibility of the activation detection between groups. Note that ρ varies between -1 and 1. When both analyses detect the exact same subset of activated voxels with matching signs (i.e., $N_1 = N_2 = A$ and $D = 0$), we obtain $\rho = 1$, which indicates full agreement. On the other extreme, when the same set of voxels are detected but with opposite signs, one obtains $\rho = -1$. If positive and negative activations are completely unrelated between two groups, then on average it is expected that the number of agreements would be equal to the number of disagreements (i.e., $A = D$), in which case

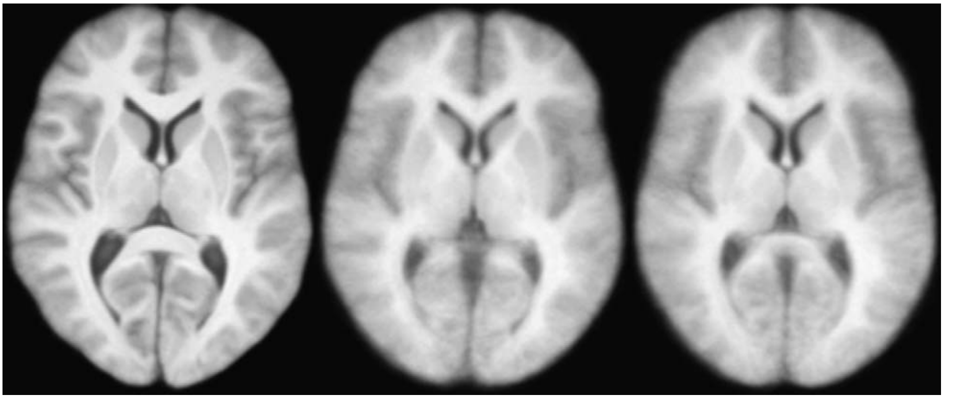


Fig. 2. A single slice from the average of 21 inter-subject registered volumes using ART (left), SPM99 (middle) and AFNI (right).

$\rho = 0$. We repeated these calculations for data processed using each of the three inter-subject registration methods. The reproducibility measures were compared using the nonparametric Mann–Whitney test.

Table 1
Post-registration landmark dispersion

Landmark	Method		
	ART	SPM99	AFNI
Frontal pole-L	1.444	2.894	3.748
Frontal pole-R	1.385	3.047	4.729
Temporal pole-L	1.534	3.745	3.637
Temporal pole-R	1.666	3.556	4.494
Anterior commissure	1.019	1.854	1.292
Posterior commissure	0.918	2.172	0.985
Corpus callosum-A	0.881	2.506	3.517
Corpus callosum-P	1.153	3.856	4.067
Frontal horn-L	1.191	2.820	3.679
Frontal horn-R	1.662	2.647	3.303
Occipital pole-L	1.451	5.318	5.282
Occipital pole-R	1.088	4.917	5.464
Putamen-R A	1.001	2.147	2.227
Putamen-R P	2.205	3.173	2.436
Putamen-L A	1.369	2.182	2.545
Putamen-L P	1.651	2.663	2.705
Caudate nucl.-R A	2.430	2.498	2.255
Caudate nucl.-L A	2.058	2.437	2.772
Lat. 4th ven.-R I	1.043	2.636	3.160
Lat. 4th ven.-L I	1.073	2.571	3.325
Mean	1.411	2.982	3.281
Standard deviation	0.436	0.903	1.187

L = left, R = right, A = anterior, P = posterior, I = inferior, Lat. 4th ven. = lateral extension of the 4th ventricle.

Table 2
Reproducibility measure ρ

Method	ART	SPM99	AFNI
Mean	0.2488	0.2290	0.2147
Standard deviation	0.01224	0.01220	0.01149

2.9. Sensitivity of activation detection

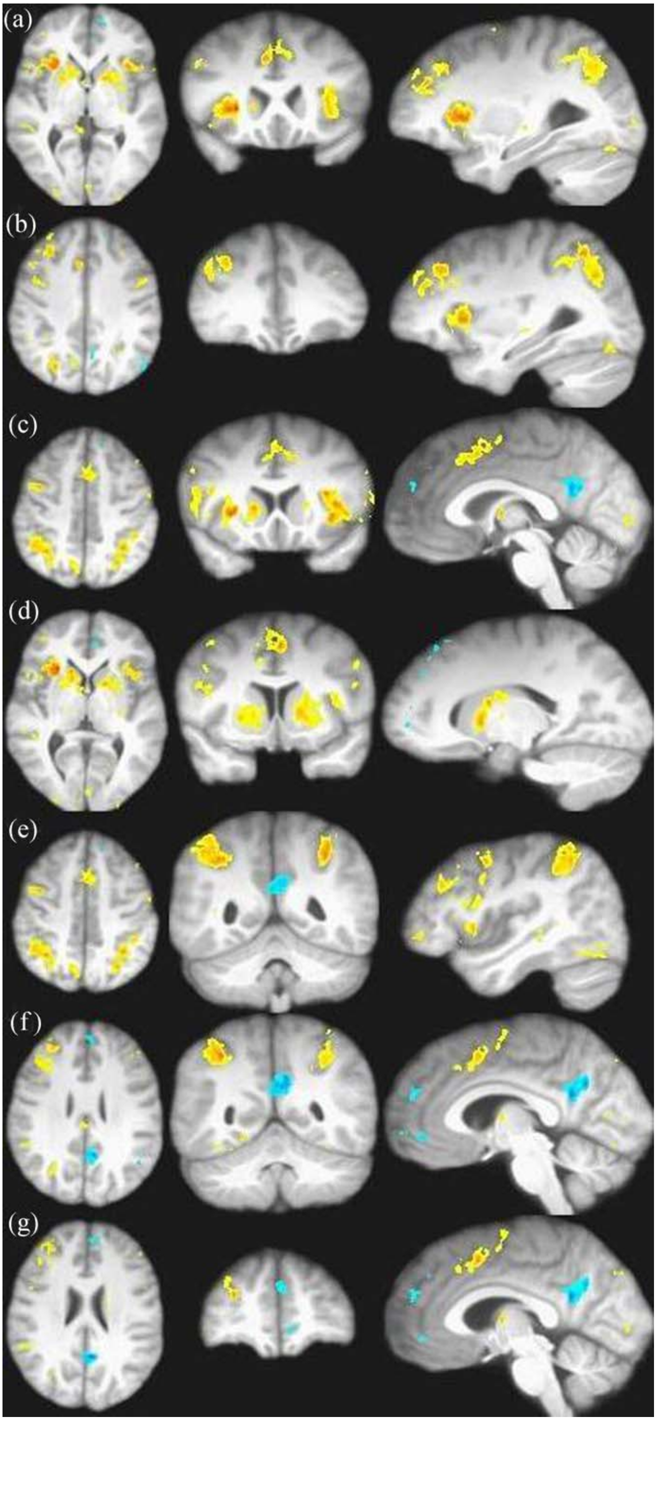
Since all the processing steps are exactly the same, except for inter-subject registration, the total number of activated voxels may be taken as an empirical measure of the sensitivity of the activation detection method. In order to statistically compare these criteria among data processed using different registration methods, we applied a procedure similar to the delete-11 jackknife method [17]. In this method, we sampled groups of 10 from the total of 21 subjects and applied our group analysis methodology. We then counted the total number of activated (positively or negatively) voxels. This process was repeated 100 times for each registration method, enabling us to examine statistical differences among the results.

3. Results and discussion

For a qualitative assessment of the accuracy of inter-subject registration algorithms, the average of the 21 registered volumes in a single slice is shown in Fig. 2. The left image is obtained by averaging the volumes registered across subjects using ART, the middle image is that of SPM99, and the right image is the average of AFNI registrations. It can easily be seen that the ART average is of a superior quality relative to the other two methods. This is due to the fact that ART is able to more accurately register corresponding anatomical entities across different subjects. Also, SPM99 appears to be slightly better than AFNI, but the difference is not obvious.

Results of the quantitative comparison based on the dispersion of post-registration homologous landmarks are shown in Table 1. The values corresponding to ART are the lowest for all landmarks, except for the right anterior caudate nucleus where AFNI's dispersion is slightly less. The nonparametric Friedman test [16] was used to assess the significance of the findings. This nonparametric test reflects the fact that the data are 'paired', i.e., the three 'treatments' are applied to the same set of individual landmarks. Friedman's $Q = 28.3$ for three groups of 20 individuals indicated significant differences between the means ($P < 10^{-4}$). A standard ANOVA yielded $F = 25.1$, so that $P < 10^{-4}$ ($df = 2$ and 57), again indicating significant differences between the means. Paired t-tests were then applied, yielding values of $t = -7.16$ with 19 degrees of freedom ($P < 10^{-4}$) for ART vs. SPM99, and $t = -6.51$ ($P < 10^{-4}$) for ART vs. AFNI, indicating that the differences between ART and each of the other programs is significant. Comparing SPM99 and AFNI, we found $t = -1.99$ ($P \approx 0.06$) suggesting that SPM99 may be more accurate than AFNI, although the result does not quite reach statistical significance.

Fig. 3. Regions of significant positive (yellow/red) or negative (blue) brain activation in the visual oddball task superimposed on group averaged high-resolution MRI.


(a)
(b)
(c)
(d)
(e)
(f)
(g)

The mean ± standard deviation of the reproducibility measure ρ for the data processed using ART, SPM99, and AFNI are given in Table 2. Although at first glance the differences between methods seem small, they are in fact significant. Nonparametric Mann–Whitney tests find the difference between the reproducibility measure of ART and AFNI to be significant at $P \sim 10^{-30}$; between ART and SPM99 at $P \sim 10^{-21}$; and between SPM99 and AFNI at $P \sim 10^{-14}$. The significance of these findings becomes more palpable when we consider the average number of co-activated voxels ($A-D$). They are 23,746, 19,376, and 17,914, for ART, SPM99, and AFNI, respectively. That is, on the average, 4370 more voxels are in agreement when two independent groups of subjects were analyzed using ART vs. SPM99, and 5832 more voxels are in agreement when ART was used vs. AFNI. Given that our voxel size is 1 mm^3, these numbers translate to a total region size of approximately 5 cm^3, which is considerable.

The summary statistics for the total number of activated voxels at $P < 0.01$, obtained from a group of 10 subjects using jackknife sampling, are (mean ± standard error): 90,675 ± 1817, 81,634 ± 1534, and 78,360 ± 1,767 for ART, SPM99, and AFNI, respectively. Mann–Whitney tests indicate that the differences between ART and the two other methods are highly significant (ART vs. SPM99: $P < 10^{-3}$; ART vs. AFNI: $P < 10^{-5}$); however, the difference between SPM99 and AFNI did not reach significance ($P = 0.089$). These results indicate that the use of high dimensional, inter-subject registration methods, such as ART, for group analysis of fMRI data can significantly increase the sensitivity of activation detection. Although all the reproducibility and sensitivity measures shown here are for activation detections at $P < 0.01$, similar results (not shown) were obtained for $P < 0.05$.

Finally, there is considerable interest in the medical and neuroscience research community on the brain activity underlying the performance of a visual oddball task. The visual oddball task activated a wide network of brain regions in our subjects. Group analysis of the 21 subjects using ART found positive activations ($p < 0.0001$) in the following regions: bilateral anterior insula at Brodmann area 45 (BA 45, Fig. 3a); right dorsolateral prefrontal cortex (DLPFC) or middle frontal gyrus (BA 9/46, Fig. 3b); bilateral anterior cingulate (BA 24/32, Fig. 3c); bilateral caudate-putamen (Fig. 3d); and inferior parietal lobule (BA 40, Fig. 3e). Negative BOLD response was observed on the left posterior cingulate gyrus (BA 23, Fig. 3f) and the left medial frontal gyrus (BA 10, Fig. 3g). In addition, we found bilateral activation on the pre-motor area (BA 6) and on the cingulate gyrus (BA 23), about 25 mm anterior to the region where the negative activation was observed. The presence of a negative BOLD signal in visual oddball detection has not been previously reported and deserves further study. The regions of negative activation remained when we randomly grouped the subjects into a group of 10 and a group of 11 individuals and analyzed each group separately. Thus, it is highly unlikely that this finding represents artifacts or type I errors.

4. Conclusions

In this work, we conducted a quantitative comparison of the registration accuracy between three registration programs: AFNI, SPM99, and ART. These are representative of low, medium, and high dimensional, inter-subject registration algorithms. Each method was used to register high-resolution, T_1-weighted MRI volumes within the same group of 21 subjects. As one might expect, the results showed that the high dimensional, inter-subject registration algorithm implemented in ART was able to reduce the anatomical variability between subjects to a greater extent as compared with low/medium dimensional transformation produced by AFNI and SPM99. Furthermore, we investigated the reproducibility of activation patterns and sensitivity of activation detection by analyzing fMRI data from a group of 21 subjects while performing a *visual oddball* task. The results indicate that the improved registration accuracy achieved by ART significantly increases the reproducibility of the activation maps and improves the statistical sensitivity of group analysis.

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