Reducing Inter-subject Anatomical Variation: Analysis of the Functional Activity in Auditory Cortex and Superior Temporal Region using HAMMER

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Abstract. Conventional group analysis of functional MRI (fMRI) data often involves spatial matching of individual data by registering every subject to an anatomical reference. Due to the high degree of inter-subject anatomical variability, a low-resolution average anatomical model is typically used as the target template, and/or smoothing kernels are applied to the fMRI data to spatially blur images. However, such smoothing can make it difficult to detect small regions such as auditory cortex when anatomical morphology varies among subjects. Here, we investigate the impact of using a high-dimensional (high-d) registration technique (HAMMER) on fMRI data analysis. It is shown that HAMMER-based analysis results in an enhanced functional signal-to-noise ratio (fSNR) with more localized activation patterns. The technique is validated against a commonly used low-dimensional (low-d) normalization (SPM2). The comparison also includes the effect of template spatial resolution, and the effect of smoothing on fSNR and on activation localization accuracy. The results demonstrate significant improvement in fSNR using HAMMER compared to conventional analysis using SPM, with more precisely localized activation foci.

1 Introduction

Inter-subject variability in the spatial location of activation foci in cognitive neuroimaging experiments is often interpreted as noise. To the extent that variability in functional anatomy reflects variability in structural anatomy, it may be decreased by improving anatomical registration across subjects. Reduction in variability in the spatial location of activation foci has several advantages: First, it increases experimental power, so that small, focal functional activations can be more easily detected. Second, it improves spatial resolution, permitting activation foci to be localized to specific anatomical locations with greater precision. A number of brain normalization techniques are used to register anatomy across subjects. These techniques vary from the linear transformation of a rigid body registration with a few parameters [1] to high degrees-of-freedom deformable registration methods [2–4]. In addition to landmark-based and intensity-based accuracy evaluation metrics, the effectiveness of inter-subject registration on the accuracy of functional group analysis has received some attention [5–7], although these published studies are relatively old now, and suffer from a number of limitations including: 1) the selected registration techniques are relatively low-d and therefore, the impact of using a high-d registration method in functional analysis has not been evaluated thoroughly; 2) the usage of low-resolution anatomical templates in current techniques overshadows the effectiveness of using a high-d inter-subject registration in group analysis; 3) the application of a spatial filter to blur/reduce inter-subject anatomical variabilities; and 4) the cognitive tasks used activate large, distributed brain networks and not focal regions, which would be superior for the assessment of the effect of high-d anatomical registration on alignment of functional data. Therefore, it would not be possible to have a clear conclusion on the effectiveness of a high-d registration technique in functional group analysis.

In this study, we investigate the effect of applying a high-d registration technique, known as HAMMER (Hierarchical Attribute Matching Mechanism for Elastic Registration), proposed by Shen *et al.* [8]. HAMMER's accuracy has previously been compared to other deformable-based registration techniques [9]. However, to the best of our knowledge, no one has examined HAMMER's performance as a normalization technique in functional group analysis of tasks yielding focal activity. We have selected a speech production and listening task as the fMRI paradigm. The functional group analysis results are validated by comparing to a commonly used low-d normalization, SPM2 (Statistical Parametric Mapping: Wellcome Department of Cognitive Neurology, London, UK). We evaluate the effectiveness of the normalization technique and the effect of the normalization template. Standard normalization techniques use a low-resolution average anatomical model as the target template and/or apply smoothing kernels to the functional data to blur images in order to compensate for inter-subject variability. Anatomical features, particularly within the extensive convolutions of the cerebral cortex, are partially blurred or completely removed. Such smoothing results in activation patterns from separate regions potentially overlapping due to the lack of a precise multi-subject registration technique (Figure 1). We compare a well-known high-resolution template ($1.0 \times 1.0 \times 1.0mm$), Colin27 or CJH27 [10], with a common average template, ICBM152 [**11**].

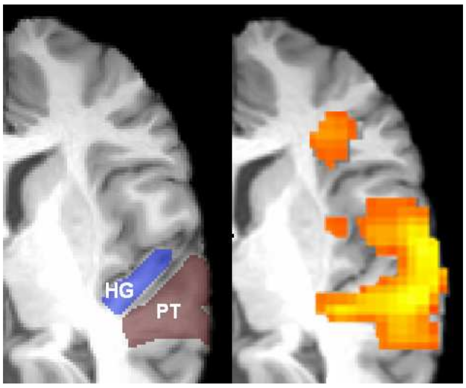

Fig. 1. Activation overlap shown for two different regions of Heschl's Gyrus (HG) and Planum Temporale (PT).

This paper is organized as follows: Section 2 provides information on data acquisition and the fMRI paradigm, preprocessing steps, and inter-subject registration. The impact of inter-subject registration on the fMRI group analysis is presented in Section 3. Section 4 contains summary and the conclusion.

2 Materials and Methods

This paper explores the application of a high-d registration technique for functional group analysis of an auditory task, and is not primarily concerned with the relevance of the results to our understanding of speech perception. Consequently, only aspects of the experimental design relevant to the methodological question are described.

2.1 fMRI Experimental Paradigm

Twenty one normal healthy volunteer subjects (16 female, 5 male, ages 23 ± 3 (mean$\pm$std), right-handed, native English speakers) participated in this study. All subjects gave informed consent to the experimental protocol, which was approved by the Queen's Health Sciences Research Ethics Board. The experiment consisted of five conditions; (a) Whispering "TED", with concomitant clear auditory feedback, (b) Whispering "TED", while hearing masking Gaussian white noise, (c) Listening to the stimuli of the first condition stimuli without speaking, (d) Listening to the stimuli of the second condition stimuli without speaking, and (e) Rest.

Conditions were presented in a pseudo-random order so that each condition appeared once in every set of five trials. Thirty-six such sets of trials were presented in each 9-minute run. Three different sequences of such trials were generated; each subject experienced each of these sequences over three runs. Here, we will concentrate on two contrasts; the first four conditions vs. rest, which should reveal activity in auditory regions concerned with speech and sound processing; and listening to speech (condition 3) compared to rest (condition 5), which should reveal auditory activity as well as activity in speech-sensitive regions of the superior temporal gyrus and sulcus.

MR imaging was performed on the 3.0 Tesla Siemens Trio MRI machine available at Queen's Center for Neuroscience Studies, Kingston. T_2^*-weighted functional images were acquired using rapid-sparse GE-EPI sequences with a typical field of view of $211 \times 211mm$, in plane resolution of $3.3 \times 3.3mm$, slice thickness of $4.0mm$, TA = $1600msec$ per acquired volume, TE = $30msec$, and TR = $3000msec$. In order to record the verbal responses without any acoustic interference, the visual cue instructing the subject to listen or speak was presented at the beginning of the $1400msec$ silent period between successive scans: trials were always complete by the end of this period. In addition to the functional data, a whole-brain 3D T1-weighted anatomical image was acquired for each participant (voxel resolution of $1.0 \times 1.0 \times 1.0mm$, flip angle $\alpha = 9°$, TR = $1760msec$, and TE = $2.6msec$).

2.2 Data Preprocessing

Structural and functional image data were preprocessed up to the inter-subject registration step using SPM2; data were motion-corrected with respect to the first volume of the first session using the realignment tool of SPM2 (i.e., 4th degree B-spline interpolation). All structural MR data were stripped to remove skull and scalp using the Brain Extraction Tool (BET) of the FSL software package (Oxford Centre for Functional MRI, Oxford University, UK) following the steps proposed by Brett [12]. Next, the structural images were rigidly registered to the functional time series using the Mutual Information Coregistration tool of SPM2. Figure 2 illustrates all the preprocessing steps up to the inter-subject registration in the form of a flowchart.

2.3 Inter-subject Registration

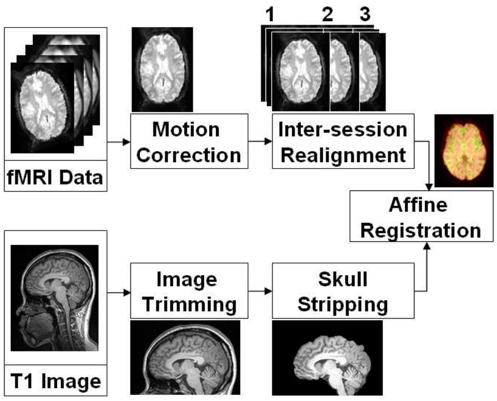

Fig. 2. Preprocessing (described in 2.2).

All 21 subjects' structural data were aligned using two registration techniques:
A) A commonly used normalization technique provided in SPM2 package was selected. Normalization in SPM was applied by using global linear (affine transform) and local nonlinear (cosine basis functions) transforms to register the structural image from each fMRI subject to: (a) a most commonly used atlas template, ICBM152 template [11], defining the so-called MNI space (ICBM152 was generated by averaging 152 T1-weighted brain volumes after affine normalization), and (b) the high-resolution $1mm^3$ Colin27 template [13]. For both cases, the registered fMRI data were smoothed using an isotropic Gaussian kernel [14] (FWHM $8mm$) to compensate for the inter-subject variability remaining after the normalization procedure. Case (a) is a standard implementation of the SPM normalization.
B) HAMMER is an elastic registration technique, which utilizes an attribute vector for every voxel of the image. The attribute vector reflects the geometric features of the underlying anatomy at different scales. Our application of the HAMMER algorithm proceeded in two steps: First, the brain data is segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) using FMRIB's Automated Segmentation Tool (FAST) of the FSL software package. Second, HAMMER registration is applied to warp the brain images to the $1mm^3$ Colin27 template. To examine the effect of smoothing, another copy of HAMMER-based normalized fMRI data was smoothed using the SPM smoothing procedure (i.e., using an isotropic Gaussian kernel (FWHM $8mm$)).
Thus, overall, we will be evaluating five different cases; (a) HAMMER-based normalization using Colin27 as the template without smoothing (HMR w/o s.), (b) HAMMER-based normalization using Colin27 as the template with smoothing (HMR w. s.), (c) SPM2-based normalization using Colin27 template without smoothing (SPMc w/o s.), (d) SPM2-based normalization using Colin27 template with smoothing (SPMc w. s.), and (e) finally, SPM2-based normalization

using ICBM152 template (SPMi). For case (e), we also considered two analyses, with and without smoothing, however, the analysis without smoothing did not reveal any activation in inter-subject random effect analysis. Therefore, we only included the results of analysis for case (e) with smoothing in this paper.

3 Results and Discussion

The accuracy of the inter-subject registration technique and its impact on the localization of activation patterns, the influence of the normalization template, and the effect of smoothing in functional group analysis were compared for the two selected registration techniques.

3.1 Structural Analysis

Normalized Cross Correlation [15] (NCC) assesses similarity between a registered volume and a reference template. The NCC of two volumetric images can be computed by first normalizing each image to have zero mean and unit variance, and then multiplying each voxel of one volume by the corresponding voxel in the other volume, and summing the products. NCC scores were computed for the entire volume for three different cases: (a) HAMMER registration using Colin27, (b) SPM-based registration using Colin27, and (c) SPM-based registration using ICBM152. In addition, given the auditory nature of our fMRI protocol, we created specific regions of interest (ROIs) around auditory cortex, extending into the superior temporal sulcus, in both hemispheres (Left: $x = -66 : -20mm, y = -50 : +15mm, z = -15 : +20mm$, Right: $x = +20 : +66mm, y = -50 : +15mm, z = -15 : +20mm$ with respect to ICBM152 coordinate frame). The results, shown in Table 1, revealed that HAMMER outperforms the SPM-based normalization, especially in highly convoluted regions such as the selected ROIs (over 8% improvement in NCC score compared to SPMc and 25% compared to SPMi).

Table 1. Comparing Mean and std. (%) of NCC values for the entire brain and an ROI around auditory cortex for all 21 subjects.

Vol.	Registration Method		
	HMR	SPMc	SPMi
	Mean±std	Mean±std	Mean±std
Entire	98.2 ± 0.1	96.5 ± 0.2	68.5 ± 0.4
L. ROI	95.2 ± 0.3	87.0 ± 1.0	60.7 ± 2.1
R. ROI	95.3 ± 0.7	87.3 ± 1.1	66.3 ± 2.2

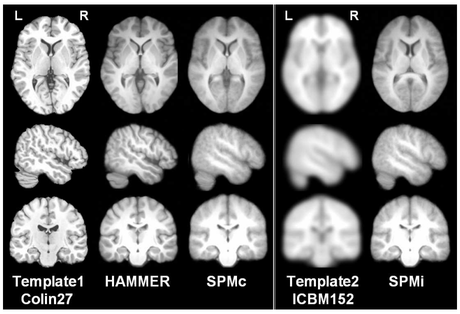

Fig. 3. Axial (z = -19mm), sagittal (x = +50mm), and coronal (y = +2mm) sections. Coordinates are in Colin27 frame.

In addition to comparing NCC scores, the mean volumes were also generated by averaging all 21 registered volumes for the three cases mentioned above.

Registration	Template	Listening vs. Rest				FirstFour vs. Rest			
		Left		Right		Left		Right	
		t value	(x, y, z) mm	t value	(x, y, z) mm	t value	(x, y, z) mm	t value	(x, y, z) mm
HMR. w/o s.	Colin27	**11.57**	-63, -24, 9	**11.28**	57, -9, -6	**10.89**	-45, -33, 18	**10.02**	57, -9, 12
HMR. w. s.	Colin27	**12.57**	-66, -36, 3	**10.81**	57, -12, -6	**11.05**	-57, -6, 27	**10.96**	57, -12, 15
SPMc w/o s.	Colin27	7.33	-51, -33, 6	6.66	63, -24, 3	8.67	-48, -12, 27	8.77	54, -9, 21
SPMc w. s.	Colin27	8.24	-51, -36, 9	7.59	66, -24, 3	10.63	-45, -12, 24	9.55	54, -6, 18
SPMi	ICBM152	8.73	-51, -36, 6	7.56	66, -24, 3	10.88	-60, -30, 9	9.13	54, -6, 24

Table 2. Coordinates of peak activation (in ICBM152 frame) given for five different cases for two different contrasts.

Figure 3 shows the cross-sections derived from the mean volumes obtained with each registration technique. It can be observed that HAMMER improves the delineation of fine features such as sulci and gyri and consequently, the spatial homogeneity between individual subject brains and the reference template.

3.2 Statistical analysis of fMRI

Statistical inference on contrasts of parameter estimates was performed with a second-level inter-subject (Random Effect Analysis or RFX [16]) model using one-sample t-tests in SPM2. The analysis was performed for the contrasts of 'Listening to speech vs. rest' and 'First four conditions vs. rest' for five different fMRI datasets: (a) fMRI images normalized using HMR w/o s., (b) fMRI data normalized using HMR w. s. (FWHM 8mm), (c) fMRI images normalized using SPMc w/o s., (d) fMRI data normalized using SPMc w. s. (FWHM 8mm), and (e) finally, fMRI images normalized using SPMi (FWHM 8mm). All analyses were performed in MATLAB® using SPM2 functions and custom coding. All analyses yielded signal activation treating subjects as a random effect in the superior temporal region bilaterally, using False Discovery Rate (FDR) correction for multiple comparisons ($p < 0.05$). The highest activation peak (i.e., t-value) in each hemisphere, and the corresponding 3D coordinates (in ICBM152 frame) are shown in Table 2.

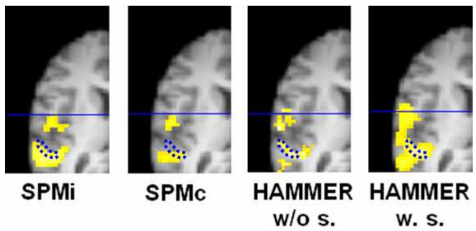

Fig. 4. Comparing activation region alignment over Heschl's gyrus. The dotted line indicates the Heschl's gyrus.

The following can be concluded from the table: First, RFX analysis of HAMMER-based normalized data, including both smoothed and non-smoothed cases, gives t-values that are 50% higher compared to SPM-based ones in both hemispheres, suggesting increased fSNR due to increased overlap across subjects. To confirm such conclusion, the Euclidean distances between the highest activation peak obtained from RFX analysis and the closest activation peak to that in each individual (obtained using Fixed Effect Analysis (FFX)) were calculated for both the HAMMER-based technique without smoothing and SPMi. The results are shown in Table 3. Comparing the results of HAMMER-based and SPMi-based analysis, it can be concluded that the

Registration	RFX loc.	Ave. Coord.(mean±std)			A.E.D.
	mm	x mm	y mm	z mm	(mean±std)mm
HAMMER w/o s.	$(-63, -24, 9)$	-62.6 ± 3.75	-25.3 ± 4.59	7.1 ± 2.80	6.18 ± 3.13
SPMi	$(-51, -36, 6)$	-54.4 ± 6.33	-33.3 ± 4.08	7.7 ± 5.44	9.37 ± 4.02

Table 3. Average Euclidean Distances (A.E.D.) between the highest activation peak obtained from RFX and the closest activation peak to that obtained from FFX.

higher t-test values resulting from RFX analysis using HAMMER registration are due to an increased activation overlap among all subjects. Second, the use of the high-resolution template with the low-d SPM normalization procedure neither increased the activation peak value nor improved the localization of activation foci. Clearly, the SPM normalization technique can not take advantage of the spatial detail in a high-resolution template to allow matching of morphologically variable regions across individuals. Third, applying smoothing kernels to fMRI data prior to group analysis does increase the peak activation in most cases. However, such smoothing degrades spatial resolution, so that activation foci cannot be localized as precisely. Figure 4 illustrates how the region of activation produced in the contrast of "first four vs rest" relates to Heschl's gyrus, the gross anatomical landmark for primary auditory cortex, across four methods. Heschl's gyrus was manually segmented on the average registered structural data, and is shown as a dotted line. The region of activation resulting from the HAMMER- based technique without smoothing aligns perfectly with Heschl's gyrus, whereas regions of activation resulting from other techniques do not align as well.

4 Conclusion

In this work, the impact of a high-d elastic registration technique, HAMMER, was investigated for group data analysis for an fMRI paradigm yielding highly focal activation. The accuracy of HAMMER was compared to that of SPM2, a commonly used low-d normalization method. NCC scores revealed that HAMMER outperformed the SPM2 normalization in inter-subject registration. Thus, in this case, a better match across subjects in brain morphology resulted in better functional signal-to-noise (higher t-statistics) and a more focal region of activation that was more precisely located with respect to primary auditory cortex. The effect of using a high-resolution template (Colin27) for normalization was also examined. The use of the high-resolution template with the low-d SPM2 normalization procedure neither increased the the t-statistics nor improved the registration of activation foci across subjects; we conclude that the SPM2 normalization technique cannot take advantage of the spatial detail in a high-resolution template to improve alignment of morphological details across individuals. Spatial smoothing was effective at increasing t-statistics and functional signal-to-noise. However, such smoothing decreases spatial resolution so that activation foci cannot be localized as precisely. Inter-individual variability in the location of activation foci across subjects has at least three components. To the extent that brains in a common reference space differ in macroanatomical structure, activation foci can be expected to be at different spatial coordinates. It is this component of functional variability that we can overcome, in part, with high-d anatomical registration. However, brains also differ in microanatomical structure, which is correlated with macroanatomy although not entirely. Thus, patches of tissue which may be microanatomically and functionally homologous across individuals may have somewhat different relationship with macroanatomical structure. This anatomical variability cannot be compensated for with high-d normalization, nor can variability in location due to the recruitment of different perceptual/cognitive processes (and thus, functionally different patches of tissue) across individuals. However, high-d registration techniques like HAMMER do provide a tool for assessing whether variability among individuals in activity for particular tasks and stimuli can be explained in part by macroanatomical variation.

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