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Measuring structural-functional correspondence: Spatial variability of specialised brain regions after macro-anatomical alignment

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ABSTRACT

The central guestion of the relationship between structure and function in the human brain is still not well understood. In order to investigate this fundamental relationship we create functional probabilistic maps from a large set of mapping experiments and compare the location of functionally localised regions across subjects using different whole-brain alignment schemes. To avoid the major problems associated with meta-analysis approaches, all subjects are scanned using the same paradigms, the same scanner and the same analysis pipeline. We show that an advanced, curvature driven cortex based alignment (CBA) scheme largely removes macro-anatomical variability across subjects. Remaining variability in the observed spatial location of functional regions, thus, reflects the "true" functional variability, i.e. the quantified variability is a good estimator of the underlying structural-functional correspondence. After localising 13 widely studied functional areas, we found a large variability in the degree to which functional areas respect macro-anatomical boundaries across the cortex. Some areas, such as the frontal eye fields (FEF) are strongly bound to a macro-anatomical location. Fusiform face area (FFA) on the other hand, varies in its location along the length of the fusiform gyrus even though the gyri themselves are well aligned across subjects. Language areas were found to vary greatly across subjects whilst a high degree of overlap was observed in sensory and motor areas. The observed differences in functional variability for different specialised areas suggest that a more complete estimation of the structure-function relationship across the whole cortex requires further empirical studies with an expanded test battery.

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Introduction

Despite the advances in functional neuroimaging of the last two decades a question which is central to cognitive neuroscience has remained largely unanswered; that is, to what extent do functional areas respect anatomical landmarks, or more generally, what is the relationship between structure and function in the human cerebral cortex? The creation of probabilistic maps based on the data from a large cohort of brains is probably the most powerful way to approach this question. One way to create probabilistic maps from a large sample of subjects is to utilise the already abundant data obtained in mapping experiments in different labs to carry out a meta-analysis. A meta-analytic approach however, would be sub-optimal as it would introduce unwanted sources of variability. Those sources are, amongst others, differences in the reporting of locus of activation due to different alignment and normalisation schemes, variability

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arising from different experimental paradigms and differences related to inconsistencies in analysis methods and thresholds used across studies (Thirion et al., 2007). In order to avoid these issues, we suggest the creation of data sets from a large cohort from which rich functional information is acquired using the same test battery and a consistent analysis pipeline.

Spatial normalisation

Spatial correspondence between subject's brains is a mandatory condition for meaningful whole brain group analysis as without accurate spatial correspondence, different cortical locations are compared across subjects as if they were the same area. Not accounting for inter-subject macro-anatomical variability is a major source of loss of statistical power in group statistics.

The most widely used normalisation procedures automatically morph an individual subject to fit a template brain. The two most commonly used volumetric normalisation procedures are a) transformation to Talairach space, based on the classic stereotactic atlas created by Talairach and Tournoux from one post-mortem examination (Talairach



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and Tournoux, 1980) or b) transformation to the MNI template space (Evans et al., 1992, 1993).

Both the Talairach co-ordinate space and MNI template system have been extremely important tools to create spatial correspondences between subjects for group analysis. These normalisation techniques provide a means by which one can compare activated brain regions across studies as results can be reported in terms of a three dimensional co-ordinate system (x, y, and z position in mm). Furthermore the widespread use of standardised co-ordinate frameworks allows for an efficient and practical system of reporting findings to the neuroimaging community and affords the possibility of creating vast databases (e.g. BrainMap.org). However a universal coordinate framework still does not exist making the creation of databases for the use of data-mining and meta-analysis difficult (Derrfuss and Mar, 2009).

(Macro-) anatomical correspondence

Despite the many benefits of Talairach and MNI registration these normalisation procedures have severe drawbacks. The major problem being that the same coordinate can refer to different anatomical areas across subjects. Fig. 1 shows two brains warped into Talairach space. One can see that, in the subject on the left, the co-ordinates x = -30 y = -25 z = 54 refer to a point on the anterior bank of the central sulcus whereas, in the second subject on the right, this same co-ordinate refers to an area on the posterior bank of the central sulcus. Even on visual inspection one can see that, despite being normalised to Talairach space, the brains still have very different cortical folding patterns. It is clear that even the most distinct macro-anatomical landmarks in the brain are often not brought into alignment after this normalisation procedure. This in turn has a detrimental effect on group statistics.

The problem of macro-anatomical alignment lies in the fact that the human neocortex is essentially a two dimensional sheet, which is only a few millimetres thick. This cortical surface is highly folded to allow it to squeeze into the restricted cranial cavity and, although there are many consistencies across subjects, each individual has a unique sulcal and gyral folding pattern. The normalisation techniques described above that align the whole brain volume by keeping it as a three dimensional box and perform affine transformations are completely blind to these individual sulcal and gyral folding patterns and thus are only able to perform a 'global normalisation' or 'registration' of the data to a common space, yet are unable to actually "align" macro-anatomical features. Whilst improvements of intensity-based volumetric alignment schemes have been proposed (Amunts and Zilles, 2001; Ashburner, 2007), approaches that explicitly align the folding pattern of the cortical surface (Fischl et al., 1999b; Goebel et al., 2006) or integrate volume and surface registration (Joshi et al., 2007, 2009) appear more promising to obtain optimal macro-anatomical alignment.

Surface-based cortex alignment

The advantages of not limiting data analysis to volume space (3 dimensional) coordinate systems but to include surface (2 dimensional) representations have been known for over a decade. Pioneering work from a number of labs demonstrated the visualisation and analytic advantages of cortical surface reconstructions (Dale et al., 1999; DeYoe et al., 1996; Fischl et al., 1999a; Goebel et al., 1998; Sereno et al., 1995; Thompson and Toga, 1996; Van Essen, 2005; Wandell et al., 2000). The creation of flat maps and inflated hemispheres from surface reconstructions of the cortical sheet meant that activity in the entire cortex, including hidden sulcal regions, could be visualised on single, canonical representations reflecting the intrinsic topology of the cortex.

The shift to 2D surface representations of fMRI data also lead to advanced alignment procedures. Early approaches of macroanatomical surface-based alignment were based on manual selection of landmarks (Van Essen, 2004, 2005; Van Essen et al., 1998). Fischl et al. showed that manual landmarks were not necessary for macroanatomical alignment, instead, a local curvature-based optimization criterion was used on spherical cortex representations (Fischl et al., 1999b). The curvature-based alignment method demonstrated much improved macro-anatomical correspondence across brains and provided first evidence of improved concomitant alignment of histiologically defined Brodman areas (Fischl et al., 2008). The use of such cortex based alignment (CBA) schemes has continued to grow and has been extended by transforming functional time course data directly in cortex space allowing one to carry out multi-subject general linear model (GLM) analysis and Independent Component



Fig. 1. These two brains, which have been transformed into Talairach space, still exhibit unique cortical folding patterns. Talairach alignment is based on a few landmarks and is unable to co register individual macro-anatomy, i.e. gyri and sulci do not perfectly match across subjects; as a result, the same co-ordinate (used to average across subjects) can refer to a different anatomical area in each subject. Here the co-ordinates x=30, y=-25, z=54 correspond to a point on the anterior bank of the central sulcus in the brain on the right. The central sulcus is indicated by a dotted line.

Analysis (ICA) directly in cortex space (Formisano et al., 2004; Goebel et al., 2006) and to register brains to existing atlases (Yeo et al., 2010).

An important examination of the effectiveness and accuracy of surface based alignment techniques was carried out by Pantazis et al. (2010). The authors compared the performance of landmark based registration, where a set of manually defined macroanatomical landmarks act as targets of alignment, and curvature based registration, which automatically extracts curvature information from each individual cortical mesh and uses local curvature deviations to drive alignment.

Landmark based alignment techniques offer more flexibility than automatic curvature-based techniques which is important in some circumstances such as when carrying out lesion studies. Even amongst brains of healthy subjects, large variation in size, shape and orientation of gyri and sulci is difficult to reconcile using automatic curvature driven alignment. Furthermore the added flexibility of landmark based registration is very useful when one considers the fact that there is not always a one-to-one relationship between the number of sulci and gyri across subjects. For example, it is well known that there is variation in the number of transverse temporal gyri across subjects, with some subjects possessing a doubling of this structure (Leonard et al., 1998).

Landmark based alignment schemes also have disadvantages however. Firstly it is very time consuming to manually label landmarks. Secondly, to do so accurately requires a significant amount of anatomical knowledge and lastly, the personal interaction in the alignment introduces the possibility of subjective labelling influencing the resulting registration. In comparison, curvature based alignment is relatively fast; as long as one starts with high quality cortical surface mesh reconstructions an automatic alignment can be completed in a matter of minutes. Another clear benefit is that it does not require detailed anatomical knowledge and that it operates largely automatically. Furthermore curvature based alignment creates reproducible results with respect to a defined objective function leaving no room for experimenter bias and error to influence the alignment.

Functional correspondence

In order to investigate the relationship between structure and function in the brain, the current project evaluates the degree of spatial correspondence of functionally homologue specialised brain areas across subjects with respect to different anatomical alignment schemes. Spatial correspondence is quantified through the creation of probabilistic maps and assessment of the geodesic distance between homologue areas. Due to the fact that volume based registration techniques are unable to account for individual differences in cortical folding and assuming there is an inherent relationship between brain anatomy and brain function, we expect a higher degree of variation in the location of functional areas when brains are registered in volume space than when the same brains are registered with advanced surface driven techniques. More specifically, if advanced curvature/landmark-based alignment largely removes macroanatomical variability (tested below) then the calculated probabilistic maps and distance measurements should largely reflect the true variability of the location of functionally defined homologue brain areas.

The common approach to solve the problem of poor overlap of functional regions when carrying out a whole-brain group study is to spatially smooth data, typically with a Gaussian kernel of 8 mm full width at half maximum. However as fMRI imaging techniques advance and data is acquired at an increasingly finer scale, smoothing in effect discards a vast amount of potentially useful data (Kriegeskorte et al., 2006). With an expected increase of spatial correspondence between functional areas across subjects, advanced surface based alignment techniques promise more powerful group statistics without the need to extensively smooth functional data. In a similar way that Fischl investigated the relationship between cortical folding and underlying cytoarchitecture (Fischl et al., 2008) the present study investigates the relationship between cortical folding and the location of functionally specialised brain areas.

In light of the potential issues of curvature-based cortical alignment (Pantazis et al., 2010) we first did a thorough investigation into our cortex based curvature alignment scheme to ensure that we significantly increased correspondence of macro-anatomical landmarks across subjects and that no major sulci were misaligned. Then functional probabilistic maps were calculated in volume (Talairach) space and aligned cortex (surface) space based on 10 subjects using 8 localisation experiments to label 13 functional areas. The obtained intriguing results motivate an extension of the project to create a freely available functional probabilistic atlas covering the whole cortex.

Materials and methods

Functional areas were localised in participants by using standard localising paradigms (see below for details). After defining specific functional regions in each subject we compared the spatial overlap of functional regions across subjects and geodesic distance of peak vertices between subjects, with respect to different normalisation/ alignment schemes. All data analysis was carried out using the Brain-Voyager QX v2.3 software package (Brain Innovation, Maastricht, The Netherlands).

Participants

10 right-handed subjects (3 females, 7 males) with a mean age of 31.5 (range 25–46 years) were scanned in 3 or 4 sessions each until the battery of 8 localising experiments had been completed for each subject. All subjects were right handed. Ethical approval was granted by the ethic committee of the Faculty of Psychology and Neuroscience at Maastricht University.

Anatomical alignment

All anatomical volumes and functional time series were first warped into Talairach space. This operation included the definition of the landmarks AC (anterior commissure), PC (posterior commissure) and the borders of the cerebrum; the defined subject-specific landmarks were then used to rotate each brain in the AC-PC plane followed by piecewise, linear transformations to fit each brain in the common Talairach "proportional grid" system (Talairach and Tournoux, 1980). Then an automatic segmentation procedure was applied leading to the creation of topologically correct cortical surface reconstructions (Kriegeskorte and Goebel, 2001). Curvature maps, which are important for mesh morphing operations, were calculated on the folded representations. All hemispheres were inflated to a sphere and underwent, separately for the left and right hemispheres, a rigid-alignment cycle where curvature of each hemisphere was aligned to a randomly chosen target subject's curvature by global rotation. The rotation parameters that produced the highest overlap between the curvature of a subject's sphere and the target sphere were used as a starting point for subsequent non-rigid cortexbased alignment (CBA).

CBA uses the extracted curvature information, reflecting the folding pattern, from each subject to create a group averaged curvature map on the sphere serving as the initial target curvature for the individual meshes. The CBA algorithm utilises curvature information as the local objective function and calculates gradient information to iteratively perform local vertex movements to increase curvature overlap. To increase robustness and accuracy, the CBA algorithm progresses from coarse to fine operating sequentially at four levels with different amounts of curvature smoothing. At the first level, individual curvature maps contain only the grossest anatomical features (central sulcus, Sylvian fissure, inter-parietal sulcus, superior temporal sulcus etc.). Each subsequent level adds increasingly more anatomical detail until finally all curvature information from the folded cortices is included. The implemented algorithm is similar to the one described in Fischl et al. (1999a,b) except that the target curvature map is calculated as the averaged curvature across all hemispheres at a given alignment stage (moving target approach, for details see Goebel et al., 2006). In this way CBA does not align each subject's curvature to a static template but to a dynamic group average that itself reflects the progress of macro-anatomical alignment.

In order to be able to ensure that CBA had improved alignment of macro-anatomical landmarks, the fundus of 26 sulci (see Fig. 2) were labelled in each subject as had been done by Pantazis et al. (2010) in their investigation of the efficacy of alignment techniques. The following sulci were identified and their courses were traced following instructions found at http://neuroimage.usc.edu/CurveProtocol.html.

To test the ability of CBA to align these landmarks we used a modified Hausdorff distance measure (Pantazis et al., 2010) to quantify the variance of pairwise path distances in the group for each landmark.

Functional localisers - area identification

Thirteen functional regions of interest were defined by performing general linear model (GLM) analysis of time course data sampled on individual cortex meshes. In order to map fMRI signal time courses from volume space to surface space, values located between the grey/white matter boundary and up to 3 mm into grey matter towards the pial surface were sampled with trilinear interpolation and averaged resulting in a single value for each vertex of a cortex mesh.

Functional areas of interest were determined by selecting the activity cluster on the surface falling in the region reported in previous standard localiser studies (see below). Once the resulting cortical patches of interest (POIs) have been marked and labelled it is possible to project the co-ordinates of a POI's vertices back into the anatomical volume in order to produce a corresponding functional volume-ofinterest (VOI) in Talairach space. Finally the POIs defined in individual cortex space can be adjusted using the transformation parameters obtained from the CBA process, resulting in functional regions in macro-anatomically aligned cortex space. The overlap of POIs across subjects can thus, be compared between volume space and (aligned) cortical surface space.

Since in the outlined procedure the extent of identified clusters – and thus the overlap calculation – depends on subjectively determined thresholds, we also implemented an alternative, completely objective method. In this approach the location of the peak vertex within the active cluster of each subject is determined per area and the shortest distance of each subject's peak vertex to the mean location of the peak vertices is calculated. The shortest distances (geodesics) of the peak vertices to the group average location were calculated on the folded group mesh using the Dijkstra algorithm (e.g. (Cormen et al., 2001) Section 24.3: Dijkstra's algorithm. pp. 595–601.).Since the distances of the peak vertices to the group average location are calculated before and after alignment, this method allows one to quantify, for each homologue area, how much CBA is able to increase spatial correspondence across subjects without requiring subject-specific adjustments of statistical thresholds.



Fig. 2. Outline of the macro-anatomical landmarks traced for each subject to evaluate the performance of curvature based alignment with respect to prominent landmarks (Pantazis et al., 2010). Note that the traced landmarks were not used during alignment; they served solely as a means to measure their inter-subject distance before and after cortex-based alignment.

Functional localisers - task battery

A battery of functional localisers was used to label functional regions across the cortex. Each POI was selected by labelling significantly active surface vertices guided by known anatomical locations. Areas in the ventral visual stream were identified using a standard localiser (Sorger et al., 2007), including the fusiform face area (FFA), parahippocampal place area (PPA) and dorsal caudal subdivision of lateral occipital cortex (LOC). The localising paradigm consisted of blocks of faces, houses, objects and scrambled objects presented for 16 s in a random order with every alternate block consisting of a rest period of central fixation. Each single stimulus was presented for 666 ms with 17 ms fixation in between each image. In all block types subjects were instructed to keep central fixation. The contrast used to localise FFA was "faces>houses". For PPA it was "houses>faces" and for LOC, "object>scrambled".

To localise V5/hMT+, an area sensitive to visual motion, a paradigm was used with two main conditions showing either 400 dots moving radially outwards from the centre of the screen or showing 400 stationary dots (Goebel et al., 1998). Four blocks of each experimental condition were presented to the subject and each alternate block contained a central fixation point and served as a block of rest. Subjects were instructed to keep central fixation during all block types. Area V5/hMT+ was identified using the contrast "moving dots>stationary dots". To functionally label the frontal eye fields (FEF), subjects were instructed to make saccadic eye movements to follow an empty white circle that moved to one of 8 possible locations on the horizontal and vertical meridian on the screen at a rate of 2 Hz for 16 s. Each alternate block consisted of 16 s of a central fixation cross. The contrast used to label FEF was "saccades>fixation".

To localise functional areas within the sensory and motor networks we used a sequential finger tapping paradigm in which subjects learnt and repeated a sequence of finger tapping movements. Blocks commenced with the presentation of five boxes in the centre of the screen. Each box corresponded with a finger digit. Subjects were cued for the first 60 ms of each block as to which hand would be used during the block with the words "left hand" or "right hand" above the boxes. Following an 80 ms delay period 4 of the five boxes lit up in a sequence with each box illuminated for 50 ms. The 2 s sequence was repeated, after an 80 ms delay, two more times so it was displayed to the subjects 3 times. During the presentation of the sequence the subjects tapped their fingers using the prescribed hand. The boxes then changed colour which cued the subject that their task was to repeat the sequence three more times without any further external cues but repeating the learnt sequence at the learnt pace. This was followed by a rest period of empty boxes which lasted 20 s. Following rest the next block commenced with the hand cue and continued with a new finger tapping sequence. In order to localise the pre-motor area (PMA) we contrasted learning phase and performance phase and labelled the most active vertices in the anatomically plausible region (i.e. anterior to the central sulcus and superior to the Sylvian fissure). To label the supplementary motor area (SMA) the contrast learning>performance was also used, however only the most active vertices on the medial surface of the hemisphere were labelled (Picard and Strick, 2001).

Using this paradigm it was impossible to differentiate between sensory and motor hand regions as the task involved both processes simultaneously. Activation anterior to the fundus of the central sulcus was labelled as hand motor area with activation posterior to the fundus of the central sulcus labelled hand sensory area.

We also employed a spatial processing/imagery task where subjects were instructed, to imagine navigating and walking around the university building when the fixation cross turned red. We chose the university building as it was very well known to each subject and provided a stimulating scene in which to navigate through. When the fixation cross turned green, subjects were asked to imagine 2-dimensional pictures, as if being shown a "national geographic-like" magazine. These were interleaved with rest periods of central fixation. All blocks were 16 s long. In order to label the precuneus, the contrast "spatial imagery>2-dimensional imagery" was used and the most active vertices on the superior parietal lobe were labelled.

To activate the language system we devised a standard word generation paradigm. A single letter was presented centrally to the subjects for 20 s and they were asked to silently generate words beginning with that letter. This was followed by a block of central fixation for an equal period of time and then a new experimental block commenced with the presentation of another letter. The contrast "word generation>rest" was used to locate two language related regions and thus labelled as "Broca's area" (anterior activations) and "Wernike's area" (posterior activations).

A cross modal region involved in the integration of input from the visual and auditory modalities was activated by simultaneous stimulation of both modalities. Sounds and images of animals were combined in 4 different ways; "congruent" blocks where the presentation of an animal image was paired with the presentation of the sound that animal makes, "incongruent" where an animal image was paired with the sound of a different animal, "images alone" where the images were presented without any accompanying auditory input and "sounds alone" where the animal noises were presented in the absence of visual stimuli (Hein et al., 2007). The contrast "congruent + incongruent> images alone + sounds alone" was used to label a multi modal region on the superior temporal gyrus (STG).

Quantifying POI overlap

We computed the overlap of functional areas across subjects for vertices belonging to a POI of at least one subject using the formula:

N_v/N .

Where N_v is the number of subjects whose functional area includes vertex v and N the number of subjects. The obtained values for all relevant vertices constitute the probabilistic map for a particular localised region-of-interest. An overall probabilistic map is obtained by adding the resulting overlap vertex values for all included POIs. A threshold of >10% is applied to all probabilistic maps to avoid depicting regions as "overlap" where only one subject has a functional area.

fMRI scanning parameters

All subjects were scanned with a Siemens 3T head only scanner (Magnetom Allegra, Siemens Medical Systems, Erlangen, Germany). A standard echoplanar-imaging (EPI) sequence (repetition time [TR] = 2 s field of view $[FOV] = 224 \text{ mm} \times 224 \text{ mm}$, matrix size $= 64 \times 64$, echo time [TE] = 60 ms) with a voxel size of 3.5 mm³. Each volume consisted of 32 slices, covering the whole brain. A high-resolution structural scan (voxel size, $1 \times 1 \times 1 \text{ mm}^3$) was also collected for each subject (in each session) using a T1-weighted three-dimensional (3D) ADNI sequence [TR, 2050 ms; echo time (TE), 2.6 ms; 192 sagittal slices]

Results

Macro-anatomical alignment results

CBA was successful in aligning most macro-anatomical landmarks. In the left hemisphere 22 of the 26 sulcal landmarks were brought closer together across subjects after CBA. In the right hemisphere 21 of the landmarks showed less variability across subjects after alignment. Landmarks with many interruptions (e.g. occipito-temporal sulcus) or which are very short (e.g. paracentral sulcus and lateral orbital sulcus) were those least well aligned (see Figs. 3 and 4). Note that all major landmarks (central sulcus, precentral sulcus, superior frontal sulcus, circular sulcus, cingulate sulcus, subparietal sulcus, calcarine sulcus, amongst others) were almost perfectly aligned, i.e. the standard



Fig. 3. Sulcal landmarks in both hemispheres before CBA on the left and after CBA on the right. Each subject is represented by a different colour. We observe that same sulci across subjects are more clustered after curvature-driven alignment as well as an increased separation between different sulci. This indicates that curvature-driven alignment is able to bring major macro-anatomical landmarks into spatial correspondence.

deviation after CBA was smaller than 3 mm. The central sulcus, calcarine, and pre-central sulcus were aligned with sub-millimetre precision.

Functional overlap results

All included functional regions showed increased overlap in the probabilistic maps after CBA compared to volume based maps. However the percent gain in overlap differed greatly across the different functional regions throughout the cortex. There remained, for example, a large amount of variability in language regions in the left hemisphere (for overview see Fig. 5). Indeed there was only a modest gain in overlap across subjects, 8.35%, when going from Talairach space, to aligned surface space in the language production area described here as Broca's region. The more posterior area activated by the language task, labelled "Wernike's area" showed a more substantial increase of 29.5% in overlap.



Fig. 4. Graph depicting the variance of the distance between the 26 sulcal landmarks used to examine efficacy of curvature driven alignment in aligning macro-anatomy in the left hemisphere (LH). CBA was successful in aligning 22 of the 26 sulci investigated.



Fig. 5. Probabilistic functional maps from across the cortex and histogram showing relative gain in overlap of functional areas after going from Talairach space to aligned cortex space. All areas show an increase in overlap, however the amount gained differs across the cortex.

Both the sensory and motor hand areas, located on either bank of the central sulcus were much better aligned after CBA. In the hand motor region there was a 102.9% gain in overlap in the left hemisphere and 91.9% gain in the same region in the right hemisphere. The hand sensory region also exhibit increased overlap with 103.9% increase in the right and 96.6% gain on the left hemisphere. Fig. 6 shows that these two distinct functional regions are blurred together in Talairach space. Projecting the data on the surface increased the distinction between the areas but it is only after accounting for individual curvature patterns using CBA that these two functional regions become clearly separated. Other motor regions exhibited smaller gains. The SMA gained 22.4% in the right hemisphere and 28.8% on the left and PMA 35.1% on the right and 68.1% on the right.

Fig. 7 highlights higher-level visual functional areas and shows the resulting increase in overlap of functional regions following CBA. Similar to results described above, CBA is able to separate functional regions which are more prone to blur together if individual anatomical curvature patterns are not accounted for. The area which shows the most gain in overlap of these regions is V5/hMT+ with 70.9% gain in the left hemisphere and 55.6% gain in the right hemisphere. Area LOC also showed increased overlap after CBA with a 62.7% gain in the left hemisphere and 38.4% on the right. Finally PPA exhibit more gain in the right hemisphere with 27.7% gain, than on the left with 17.6%.

The precuneus, activated during the spatial navigation task, showed a surprising disparity of the extent of the gain obtained in overlap between volume and CBA space. On the right hemisphere there was a very small 3.7% gain, whilst the left hemisphere showed 58% increase.

One of the most intriguing findings of a functional area that shows a strong correspondence with a macro-anatomical landmark is the frontal

eve fields (FEF). This area has been shown to be involved in primate (Bruce and Goldberg, 1985) and human (Petit et al., 1997) saccadic eve movements. We consistently found an area of significant activation at the junction of the precentral sulcus and the superior frontal sulcus as described by Paus (1996). Overlap increased by 66.7% in the left hemisphere and 106.5% in the right hemisphere. Fig. 8 shows the spatial extent of FEF in 10 subjects in the probabilistic functional map; the brighter the colour, the higher the number of subjects that contain FEF at the respective position (vertex). The left side shows the group overlap map before any cortex based macro-anatomical alignment. Although all 10 subject's FEFs occupy a coherent and anatomically plausible patch of cortex, there is not much overlap across subjects. This is indicated by the solid brown colour. The image on the right shows the same 10 subject's FEFs after the functional data have been transformed in aligned cortex space. It is clear that there is significantly more overlap after alignment with increasing overlap indicated by increasingly bright colours. The white colour at the centre of the patch indicates that FEF is located at this region in all subjects of the group. The image also depicts the sulcal paths; although these macro-anatomical landmarks were not explicitly aligned, curvature-based alignment has co-registered them successfully (different colours indicate landmarks of different subjects). Since macro-anatomical variability has been almost completely removed in this location, the high degree of functional overlap suggests a tight correspondence between structure and function in this area.

The fusiform face area (FFA) on the other hand, does not exhibit the same strong structural-functional correspondence and saw more modest increases in overlap after macro-anatomical alignment with 44.1% and 12% gain for the left and right hemispheres, respectively. The FFA is a functional area which receives much attention



Fig. 6. Probabilistic maps of the hand-motor (red) and hand-sensory (blue) areas in Talairach space (above) projected onto the surface (middle) and after curvature driven surface alignment (below). Brighter colour indicates increased overlap.

due to its role in face processing (e.g. Grill-Spector et al., 2004; Kanwisher et al., 1999; Tarr and Gauthier, 2000; Weiner and Grill-Spector, 2010). Its location showed a very interesting pattern across subjects. As seen in Fig. 9, there is much more inter-subject variability than in FEF. After curvature-driven CBA however, the differences mostly lie along the length of the fusiform gyrus and not in the direction perpendicular to that gyrus.

Peak vertex results

In the left hemisphere all functional areas showed substantial reduction of the distance of the peak vertices to the respective group average (centre) location with FEF again exhibiting the largest gain from unaligned to aligned cortex space. Most functional areas in the right hemisphere also showed a reduction of the distance of the peak vertices to the respective group centre location, albeit less substantially. Two areas, PPA and SMA, exhibited a very slight increase in the mean distance to the group average location (see Fig. 10).

Discussion

By substantially reducing macro-anatomical variability of the cortex through curvature driven surface alignment we have been able to reveal the underlying relationship between structure and function for a number of specialised functional brain regions.

In order to investigate structural-functional correspondence through the creation of probabilistic functional maps, it is crucial to achieve whole brain macro-anatomical correspondence across subjects. Our careful examination of the efficacy of CBA has shown that automatic curvature based alignment techniques can align landmarks with very high accuracy. In fact, curvature driven alignment may even outperform landmark-based cortex alignment in achieving overall correspondence as alignment is not limited to a subset of regions (landmarks) but rather utilises curvature information from the entire cortical sheet to achieve alignment. It is imperative, however, that the results of an automatic alignment be examined and not just assumed to have produced an accurate group alignment. By assuring for each subject that the used curvature-driven CBA has correctly aligned all major macro-anatomical landmarks, one can interpret the observed



Fig. 7. Zoomed view on probabilistic functional maps of major visual areas in Talairach space (top), non aligned surface space (middle) and CBA aligned cortex space (bottom). Green = PPA, Red = FFA, Blue = LOC, Orange = V5/hMT+. Brighter colour indicates increased overlap.

spatial variability of functional areas as mainly a measure of structuralfunctional relationship. Those sulci that did not seem to exhibit a reduction in variability after CBA were also those that were difficult to label, including very short landmarks (paracentral sulcus), or sulci located very close to parallel sulci (lateral orbital sulcus). It is thus possible that the macro-anatomy in these respective regions is, in actual fact, well aligned but this not reflected well due to mislabelling. This highlights the difficulty in labelling landmarks, especially those with multiple interruptions. The gain in overlap of functional areas that are located close to such landmarks are likely underestimating the true gain and should be considered preliminary. Our current set of functional areas were, however, located in regions where the surrounding macro-anatomy was aligned well (with the exception of Broca and, to some extent, FFA)

Our data show that there is a surprising amount of variability in that not all functional areas are tightly bound to anatomical landmarks, i.e. there is no general rule describing how macroanatomical and functional areas correlate. Indeed in some regions there is a strong structural-functional correspondence whilst in others the spatial location of the functional area varies greatly across subjects within a cortical area. The FEF and FFA seem to be interesting examples of this observation. Whilst it seems that CBA has brought



Fig. 8. Frontal eye fields in 10 subjects. Left before CBA alignment, right after CBA alignment. There is much more overlap of the location of the functional area after macroanatomical alignment (seen with a brighter colour). This suggests that there is a strong relationship between structure and function in the FEF.

the functional area FEF into alignment, there is a substantial amount of variability in FFA This remaining variability in the spatial location of FFA is likely to be caused by two factors. Firstly, because CBA aligns macro-anatomical curvature, there is much less alignment force along the length of a sulcus or gyrus (rather constant curvature values) than perpendicular to its extension (varying curvature values) leading to less good alignment of FFA along the fusiform gyrus. Indeed Fig. 11 shows that the anterior to posterior variability remains after CBA whilst the medial to lateral variability has been reduced substantially by macro-anatomical alignment. Another explanation for the remaining variability in the case of FFA may be that there is not one single area active in the fusiform gyrus when running a standard FFA localiser and that a different sub-region, activated by the FFA localising paradigm may be selected as "FFA" in different subjects (see also Weiner and Grill-Spector, 2010). This would indicate that the observed variability along the fusiform gyrus reflects micro-anatomical (i.e. cytoarchitectonic) differences (Eickhoff et al., 2005) which are unobservable in vivo. Indeed published probabilistic cytoarchitectonic maps show



Fig. 9. Probabilistic map of FFA before alignment (left) and after alignment (right) with sulcal paths superimposed. Anatomical alignment has brought the functional areas together however there still remains variability along the length of the gyrus.





Fig. 10. Histogram showing distance to the mean vertex location before and after alignment. Overall the peak active vertex of each subject showed movement towards the group mean after alignment reflecting the finding that the macro anatomical alignment results in concomitant improvements in the alignment of functional areas.

similar variability in spatial extent and location of micro-anatomically defined areas. In particular the spatial location of BA 44, functionally defined as "Broca's area" has been shown to be highly variable (Amunts et al., 2004), even when individual curvature has been taken into account (Fischl et al., 2008) which supports our findings. A similar investigation into the variability of hOc5, thought to be the micro-anatomically defined area which is functionally known as hMT+, also shows a similar variability profile as the map of hMT+ in the present study (Malikovic et al., 2007).

We also observed greater overlap of functional areas (and stronger reduction of peak vertices to respective centre locations) in the left than in the right hemisphere. The number of subjects (10) is too small to draw definite conclusions about a strong asymmetry and the reasons for this observed difference. It might be that functional localisers do not work as well in the right hemisphere than in the left, or that macro-anatomical alignment does not work as well as in the left hemisphere, or that the structural–functional relationship is weaker in the right hemisphere.



Fig. 11. Fusiform face area in 10 subjects. Each colour represents FFA in one subject. Despite the fact that CBA has aligned the fusiform gyrus well in the medial-lateral direction, there still remain differences in the spatial location of the FFA along the length of the gyrus (anterior-posterior), where there is little alignment force. Macro-anatomical alignment alone is not capable of resolving these differences that are most likely to reflect differences of cytoarchitecture (i.e. different active regions might be labelled as "FFA").

By creating probabilistic functional maps using the same subjects for the investigation of all functional areas, the same scanner and the same analysis pipeline we have been able to gain important insights into the extent to which macro-anatomical landmarks are coupled to specific functional areas. A limitation of the current approach is that, due to individual differences in level of activation, it is impossible to use the same threshold for every subject. For this reason, great care has been taken to individually inspect all functional data and appropriately select the extent of each and every functional region. It appears however that region selection, as currently implemented, accurately reflects the underlying functional regions as the results of the gains in functional overlap are mirrored by the decreases in the distance of peak vertices to the group average location of an area. Since the latter measure is obtained without subjective choices, it provides converging evidence that macro-anatomical alignment substantially improves functional spatial correspondence in many areas.

One must also note that other sources of spatial variability may remain in the present probabilistic maps, one of which may be intrasubject variability. In short intra-subject variability is the slight variance in location of activation observed in one subject, under the same experimental conditions, across separate scanning sessions. It has been reported that intra-subject variability, as examined by a set of localising paradigms of high level visual areas, was on a subvoxel level (Peelen and Downing, 2005). More recently however Duncan et al. (2009) indicated that the spatial location of functional areas can vary slightly within the same subject across sessions but notes that intra-subject variability can be minimised by using relaxed thresholds. The current study, by utilising robust localisers of well studied areas and not applying strict thresholds, should have kept intra-subject variability at a minimum according to these previous studies.

Our current study has important implications for statistical wholebrain analysis of group fMRI studies since our results indicate that functional correspondence is substantially improved in most investigated specialised areas. The application of an advanced surface-based macro-anatomical alignment scheme thus substantially increases statistical power beyond what is achievable with standard affine/piecewise linear volume normalisation techniques. It will be also interesting to assess in the future whether advanced volumetric normalisation schemes (e.g. Ashburner, 2007) lead to increased overlap of functionally defined specialised areas.

Despite substantial improvements, differences still remain in the location of functional regions after curvature-based alignment however, indicating that not all functional areas are tightly bound to anatomical landmarks (see Figs. 5, 8 and 9). Depending on the research question, it may be advantageous to forego anatomical accuracy and include functional landmarks as additional targets of alignment. Just as CBA uses curvature information as targets of alignment, functionally driven CBA (fCBA) would include functionally defined landmarks in the alignment procedure (Frost and Goebel, 2010). It must be noted however that fCBA would distort the anatomical information thus questions concerning the relationship between structure and function cannot incorporate this procedure, i.e. its use should be limited to further improve group analyses combining the advantages of whole-brain and ROI analyses. A similar procedure has been carried out using distributed networks as targets of multi-subject registration (Sabuncu et al., 2010). Registration based on functional data has also been used to create highly accurate and clear delineations of early visual areas (Larsson and Heeger, 2006). We hope that our present study, quantifying the structural-functional relationship in several areas located across the cortex, will help to reveal for which functional areas fCBA would be of benefit. So far it seems that areas such as FEF do not require this additional alignment step; however areas such as FFA would certainly benefit in cases where group spatial correspondence and improved statistical power is of more interest than anatomical accuracy.

Conclusions

Since our analysis suggests that there is no general level of structural-functional correspondence, it is important to map more functional brain regions. We will continue to not only add more functional areas to our map but also more subjects. With the resulting extensive database we will create a probabilistic functional atlas across the whole cortex that will be made freely available to the neuroimaging community¹ in BrainVoyager format as well as in the GIFTI format (http://www.nitrc.org/projects/gifti) that is supported by all major software packages. Aside from basic insights in the structural-functional relationship of many specialised areas, such a probabilistic functional atlas may also serve as a useful tool to aid in labelling functional areas.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10. 1016/j.neuroimage.2011.08.035.

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¹ A preliminary probabilistic functional atlas based on data gathered so far will be provided on request. For more details please contact the corresponding author.

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