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Relating connectional architecture to grey matter function using diffusion imaging

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Understanding brain function in terms of connectional architecture is a major goal of neuroimaging. However, direct investigation of the influence of brain circuitry on function has been hindered by the lack of a technique for exploring anatomical connectivity in the *in vivo* brain. Recent advances in magnetic resonance diffusion imaging have given scientists access to data relating to local white matter architecture and, for the first time, have raised the possibility of *in vivo* investigations into brain circuitry. This review investigates whether diffusion imaging may be used to identify regions of grey matter that are distinct in their connectional architecture, and whether these connectional differences are reflected either in local cytoarchitecture or in local grey matter function.

Establishing a direct relationship between regional boundaries based on diffusion imaging and borders between regions that perform different functions would not only be of great significance when interpreting functional results, but would also provide a first step towards the validation of diffusion-based anatomical connectivity studies.

Keywords: grey matter parcellation; diffusion MRI; tractography; structure/function

1. INTRODUCTION

The relationship between brain structure and brain function is fundamental to neuroscience. Early studies designed to parcellate human and non-human cortex into regions that were distinct in their structure (Brodman 1909; Vogt & Vogt 1919) acted under the hypothesis that these structurally defined regions also differed in their function. Such studies parcellate cortical regions on the basis of microstructural features such as cyto- or myeloarchitecture (Vogt *et al.* 1995; Roland & Zilles 1998; Zilles & Palomero-Gallagher 2001), which have direct implications for local cellular processing. Cortical regions, which may be delineated on the basis of cellular microstructure, have also been shown to differ in their connections to other brain regions (e.g. Jones & Burton 1976; Vogt 1993).

Independent of their respective relationships to cellular microstructure, there is good reason to expect a strong correspondence between regional brain function and connectional architecture. Anatomical connections constrain the nature of the information available to a region and the influence that it can exert over other regions in a distributed network: local structural organization can therefore be expected to *determine* local functional specialization. However, this relationship between circuitry and function has proved difficult to test directly in the absence of a mechanism for investigating connectivity in the functioning brain. Direct information relating to anatomical connections in the human brain is sparse and, until recently, only available post-mortem. More direct assessment of connectivity is feasible using invasive tracer techniques in non-human animals, but these approaches must be focused on specific regions, and assessment of function in the same animals is technically demanding (although excellent examples do exist; Yoshida *et al.* 2003).

Magnetic resonance diffusion imaging is able to characterize the local diffusion properties of water in tissue at a millimetre scale (Le Bihan 2003). In tissue with a high degree of directional organization, the self-diffusion of water is hindered more in some directions than others. For example, water diffusion in brain white matter is more hindered perpendicular than parallel to the major axis of a fibre bundle (Moseley *et al.* 1990), such that the direction of least resistance to diffusion (or principal diffusion direction) aligns well with the mean fibre orientation in an imaging voxel (Basser *et al.* 1994). Recent developments in diffusion tensor imaging techniques have enabled this diffusion *anisotropy* to be quantified, and for the principal diffusion direction to be computed at each voxel. Using these local fibre orientations, early studies were able to trace large white matter fibre tracts (Basser & Pierpaoli 1996; Mori *et al.* 1999; Catani *et al.* 2002), raising the possibility of explorations of anatomical connectivity in the living human brain. Using *diffusion tractography*, it has been possible to identify specific patterns of anatomical connectivity associated with grey matter 'seed' voxels (Conturo *et al.* 1999; Behrens *et al.* 2003*a*), and to demonstrate that spatially near, but functionally distinct, grey matter seed voxels have very different connectivity patterns (figure 1).

In this paper, we investigate whether it is possible to use diffusion-based connectivity information in a systematic fashion in order to parcellate grey matter according to its connectional architecture, and hence

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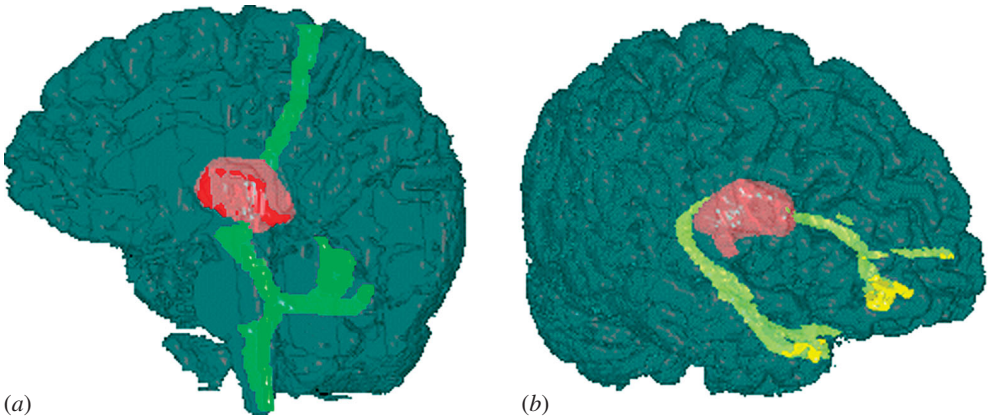
One contribution of 21 to a Theme Issue 'Multimodal neuroimaging of brain connectivity'.



(*a*) (*b*)

Figure 1. Tracing anatomical connections with diffusion imaging: (*a*) seeded from a single voxel in a ventral lateral location in thalamus. In macaque monkeys, ventral lateral nucleus of thalamus (VL) processes motor information and projects to primary motor cortex (M1; Jones 1985). The connectivity distribution both ascended to the anterior bank of the central sulcus (M1) and descended. The descending distribution followed two distinct paths, one entered the cerebellum and branched, terminating in the cerebellar cortex, the other continued further down the brainstem. (*b*) Seeded from a single voxel in a medial dorsal location in thalamus. In macaque monkeys, mediodorsal nucleus of thalamus is associated with cognitive processing, and is reciprocally connected to prefrontal (PFC; Tanaka 1976) and temporal cortices (Markowitsch *et al.* 1985). The connectivity distribution progressed anteriorly to the lateral prefrontal cortex and also, at first posteriorly, around the posterior edge of the thalamus, and then anteriorly to the anterior temporal cortex. In each case, the thalamic seed voxel is strongly connected to cortical regions relevant to its function. The two seed voxels, which are functionally distinct, may also be distinguished on the basis of their connectivity distributions. Based on Behrens *et al.* (2003*a*).

infer boundaries between discrete *functional* regions. We overview three possible approaches for parcellating the brain on the basis of diffusion MRI. These strategies differ not only in their technical approach to identifying boundaries, but also conceptually in the information they consider.

The first two applications aim to identify distinct regions in human thalamus (Behrens *et al.* 2003*a*; Wiegell *et al.* 2003). In Behrens *et al.* (2003*a*), prior knowledge of the major cortical connection sites of the thalamic nuclei in non-human primates is used to identify regions in human thalamus by associating thalamic seed voxels with their most probable cortical projection targets, as estimated by diffusion tractography. In Wiegell *et al.* (2003), thalamic nuclei are identified with no prior knowledge of their patterns of connectivity, discriminating instead on the basis of voxel-wise diffusion properties. Regions are effectively distinguished on the basis of the local properties of white matter fibres as they penetrate the thalamic grey matter.

In the third study reviewed here, the focus is on a region of cortical grey matter—medial area 6 (Johansen-Berg *et al.* 2004). White matter penetration in neocortex is substantially weaker than in subcortical structures such as thalamus; hence, in comparison to such structures, local diffusion properties are relatively homogeneous across cortical voxels, and tracking anatomical connections from cortical voxels to their remote cortical targets is relatively unreliable. Moreover, the evolution of neocortex is such that it is often difficult to make accurate predictions about the connectivity patterns from human cortical regions by examining only data from non-human primate. Here, supplementary motor area (SMA) and preSMA are distinguished with no prior knowledge of their connectional architecture by searching for a sharp *change* in the entire profiles of connectivity from the seed locations. The acting hypothesis is that, if the SMA and the preSMA perform different functions, they must require *different* connectional architecture, so that by searching for the boundary where this architecture changes, the functional border may be inferred.

2. CONNECTIVITY-BASED PARCELLATION OF GREY MATTER

(a) *Parcellation according to most probable remote cortical targets*

Functionally distinct grey matter regions have different patterns of remote connectivity. In some cases, these connectivity differences are well established from animal studies and post-mortem investigations. For example, the specific cortical connectivities of cytoarchitectonically distinct thalamic nuclei are reasonably well described (Tobias 1975; Tanaka 1976; Jones *et al.* 1979; Yarita *et al.* 1980; Jones 1983, 1985; Markowitsch *et al.* 1985; Russchen *et al.* 1987). We previously used probabilistic diffusion tractography (Behrens *et al.* 2003*b*) along with prior knowledge of the characteristic cortical projection sites of the specific nuclei, to associate seed voxels in thalamus with their preferred cortical projection site and, therefore, with their most likely thalamic nucleus (Behrens *et al.* 2003*a*; figure 2). Probabilistic tractography is seeded from each thalamic voxel and the probability of connection from that voxel to predefined cortical target regions are recorded. Thalamic voxels are classified according to the cortical region with which they have the highest probability of connection on the basis of probabilistic diffusion tractography. As expected, thalamic seed voxels form into clusters with common cortical targets (figure 2*a*(iii)). On the basis of the strong correspondence between these clusters in the human thalamus, known locations of human thalamic nuclei (Morel *et al.* 1997), and connections in non-human primates (Jones 1985), we propose that these clusters correspond to different thalamic nuclei or

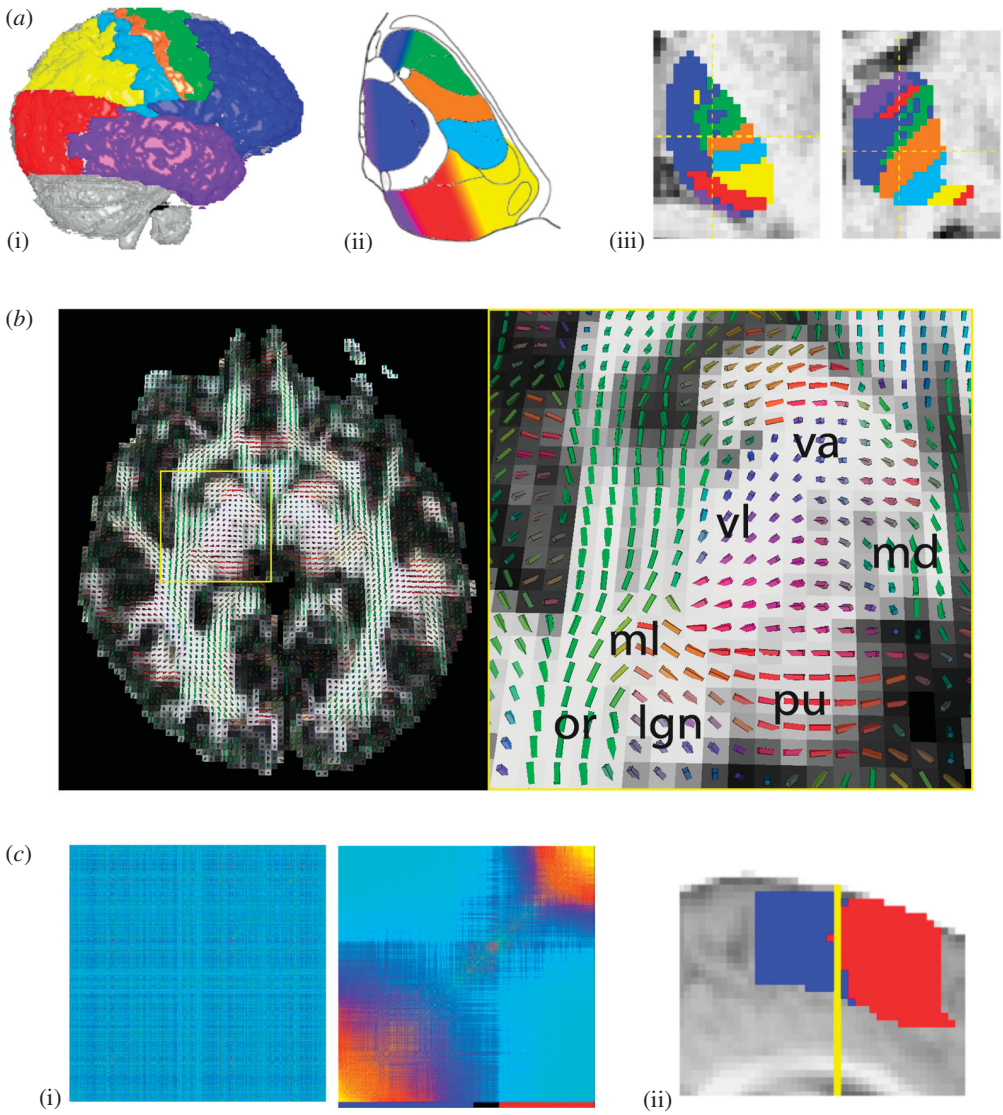


Figure 2. Diffusion-based parcellation of grey matter: (*a*) Identifying thalamic nuclei on the basis of their remote cortical projections. (i) Cortex was first segmented into large anatomically defined regions corresponding to known connection areas of the major thalamic nuclear groups in non-human primates. Subsequently, probabilistic tractography was seeded from each thalamic voxel, and the probability of connection from that voxel to each of the cortical regions was recorded. (ii) Shows an axial section based on a histological atlas of the human thalamus with nuclei outlined by black lines (Morel *et al.* 1997). Nuclei have been colour-coded according to the cortical zone with which they are expected to show the strongest connections on the basis of the non-human literature. (iii) Shows axial (left) and coronal (right) sections through a human thalamus in a single subject, with each thalamic voxel colour-coded according to the cortical zone with which it had the highest probability of connection. (figure based on Behrens *et al.* 2003*a*). (*b*) Identifying thalamic nuclei on the basis of their local diffusion properties. Left: cuboid rendering of diffusion tensors in a single slice through a human brain. Cuboids are coloured according to the orientation of their principal axis. Red corresponds to medial–lateral; green to anterior–posterior; blue to superior–inferior. Right: close-up through thalamus. Clusters are identified according to the diffusion tensor in each voxel. Nuclei labels were assigned based on the location and fibre orientation of the clusters (Wiegell *et al.* 2003). The lateral geniculate nucleus (lgn; purple) bends though Meyer's loop (ml; purple turning green) to the optic radiation (or; green). The pulvinar nucleus (pu; red) projects medial-laterally and the mediodorsal nucleus (md; green) anteriorly. The ventral lateral nucleus (vl; blue-purple) projects superior-laterally and the ventral anterior nucleus (va) superiorly. Courtesy of Ulas Ziyan (MIT) and David Tuch (Harvard Medical School and Massachusetts General Hospital). (*c*) Identifying the boundary between SMA and preSMA on the basis of a sharp change in connectivity profile. Connectivity profiles are seeded from every voxel in an area including SMA and preSMA. The correlation between each pair of profiles is stored in a matrix ((i) left) whose nodes are reordered according to a spectral reordering routine (Johansen-Berg *et al.* 2004). Groups of commonly connected clusters are clearly visible in the reordered matrix ((i) right) and are identified by the colour bar below the matrix. These clusters map back to spatially contiguous regions of cortex aligned along the anterior–posterior axis. The division between the clusters is close to the vertical line from the anterior commisure ($y=0$ mm in MNI space; Evans *et al.* 1992—yellow line in (ii))—the best approximation for the division between human SMA and preSMA (Zilles *et al.* 1996). (Figure based on Johansen-Berg *et al.* (2004), © 2004 the National Academy of Sciences.)

nuclear groups (Behrens *et al.* 2003*a*). For example, the medial dorsal cluster with a high probability of connecting to prefrontal and temporal cortices is likely to correspond to the mediodorsal thalamic nucleus which is reciprocally connected to the prefrontal (Tanaka 1976) and temporal lobe (Markowitsch *et al.* 1985). More laterally, there is a strip of clusters aligned along the anterior–posterior axis with a high probability

of connecting to: premotor cortex (most anterior), primary motor cortex and somatosensory cortices (most posterior). These clusters are likely to correspond to the ventral anterior nucleus that connects to premotor cortex, the ventral lateral nucleus that connects to primary motor cortex and the ventral posterior nucleus that connects to somatosensory cortices (Jones 1985).

Although the correspondence between the clusters identified from connectivity data and histological sections of human thalamus is compelling (figure 2*a*(ii,iii)), the precise thalamic borders identified using diffusion data in this way depend on the borders that are initially manually defined on the cortex. This dependence is not ideal for a number of reasons. First, the correspondence between gross anatomical landmarks underlying cortical cytoarchitecture is debatable (e.g. Amunts *et al.* 1999). Also, the parcellation depends on prior knowledge of what constitute meaningful connectivity target regions. This is reasonably well achieved in the case of the thalamus, as thalamo-cortical relationships are well described, but may be more challenging for other regions.

(b) *Parcellation according to local diffusion properties*

One solution to the problem of having to prespecify meaningful target regions is to parcellate grey matter simply on the basis of local diffusion properties. This approach was taken by Wiegell *et al.* (2003), who also aimed to subdivide the human thalamus. Parcellation, according to local diffusion properties, does not rely on *a priori* anatomical knowledge, or on the explicit estimation of the connectivity patterns of thalamic voxels. The authors hypothesize that differences between nuclei in their remote anatomical connectivity pattern may be seen as differences between nuclei in the characteristic orientation of the cortico-thalamic/thalamo-cortical striations within the nucleus. Hence voxels belonging to different thalamic nuclei should be distinguishable on the basis of their local diffusion properties alone.

The diffusion tensor is reconstructed at each thalamic voxel (figure 2*b*) and a metric is defined that allows the calculation of the dissimilarity or *distance* between any two diffusion tensors. The authors submit these diffusion tensor data to an automated clustering routine that attempts to partition the thalamus into groups of voxels that are close, both spatially and in terms of their diffusion tensors. Tensors are labelled as 'close' to one another if they are similar in properties such as diffusion anisotropy and mean diffusivity and if their principal orientations are well aligned. The resulting clusters (labelled in figure 2*b*) bear close resemblance to the connectivity-defined regions in figure 2*a*, and to previously reported histological segmentations of the thalamic nuclei. For comparison with the approach described in the previous section, again note the medial dorsal and anterior clusters of voxels whose principal axis of diffusion is in the anterior–posterior orientation (green), pointing towards prefrontal cortex, and note the lateral cluster of voxels whose principal axis is in the inferior–superior orientation (blue/purple) pointing towards sensorimotor cortices.

Parcellation with respect to local diffusion properties could provide a powerful means of subdividing grey matter. One strength of the technique is that it does not require prior specification of connectivity targets, and does not rely on the accuracy of diffusion-based tractography routines. However, the approach does rely on differences in remote connectivity being visible as differences in local diffusion properties. Cases will exist in which voxels with similar local diffusion properties (e.g. similar local fibre orientation) have divergent patterns of remote connectivity. In such cases, local information alone will not be enough to identify the connectional differences.

(c) *Parcellation on the basis of sharp changes in connectivity*

We have seen that by examining diffusion data relating to either remote cortical connectivity or local fibre orientation it is possible to identify distinct regions in the human thalamus that are proposed to correspond to functionally distinct thalamic nuclei. However, the techniques used to identify these divisions may not be easily generalizable to different regions in grey matter. Specifically, thalamus is a subcortical grey matter structure in which there is a large degree of white matter penetration. Local diffusion orientations within thalamus are well defined and heterogeneous. Diffusion tractography seeded from thalamic voxels is robust and accurate. By comparison, cortical grey matter regions have a much lower degree of white matter penetration, hence diffusion orientations are less well defined and local diffusion properties in general are more homogeneous. At least partly as a result of this, diffusion tractography can be less reliable when seeded from cortical rather than from subcortical grey matter. Moreover, connectivity-based approaches relying on predictions from non-human primate data cannot be applied to human cortical regions with no unequivocal homologue in other species.

In Johansen-Berg *et al.* (2004), we propose a fundamentally different strategy for inferring structural parcellation from diffusion data that allows 'blind' discrimination of regions with different patterns of connection, and which benefits from the sensitivity gained by considering a seed point's remote targets without having to rely on a complete, or even accurate, representation of its connectivity. Instead of classifying a single seed point on the basis of its cortical target, we consider the entire 'profile' of connectivity from all seed points simultaneously. This allows us to find clusters of seed voxels with very similar patterns of connectivity or, equivalently, draw boundaries between adjacent brain areas whose patterns are markedly different.

The specific cortical focus in this paper is medial area 6—an area which provides an excellent test case. In macaque monkey, the medial part of the homologue of Brodmann's area 6 consists of two cytoarchitectonically distinct regions (Vogt & Vogt 1919; Matelli *et al.* 1991), thought to correspond to SMA proper and preSMA. These two regions exhibit different functional responses (Matsuzaka & Tanji 1996), have distinct connections (Luppino *et al.* 1993) and may be distinguished on the basis of gross anatomy as lying on the medial surface, either

side of a plane approximately at rostro-caudal to the level of the bow of the arcuate sulcus. In the human brain there is consistent evidence for a functional distinction, at least between anterior and posterior parts of medial area 6, as functional imaging studies have found differential involvement of these regions in tasks engaging distinct cognitive or motor domains (Picard & Strick 1996; Rushworth *et al.* 2002). However, there is no local anatomical landmark that differentiates functionally defined SMA and preSMA in the human brain; the vertical line from the anterior commissure (VCA line) provides the best approximation (Zilles *et al.* 1996).

We aim to find a connectional dissociation within medial area 6 which corresponds to the known functional dissociation between SMA and preSMA. The hypothesis is that voxels within either one of the functionally dissociated regions will have very similar patterns of connectivity, but that there will be a sharp transition in connectivity at the functional boundary, such that the characteristic patterns will be very different between the two regions.

Connectivity distributions are seeded from voxels in a region in medial frontal cortex that includes both functional areas. Every connectivity distribution is correlated with every other, and the correlations between each of these pairs of distributions are stored in a matrix (figure 2*c*(i)). Each element in this matrix, therefore, represents the similarity between connectivity profiles of two specific medial frontal seed points. If the hypothesis of a connectional dissociation is accurate, then this matrix will contain high values at locations corresponding to the correlation between two voxels in the same brain region (demonstrating the similarity of their connectivity patterns), whereas the correlation between connectivity patterns originating from voxels in different brain regions should be low. In its original form, it is not possible to identify such patterns in the correlation matrix, as seed voxels are entered along the axes of the matrix in a random order. To test for this organization, the data are submitted to a spectral reordering algorithm (Johansen-Berg *et al.* 2004), which reorders the rows and columns of the similarity matrix, such that large element values in the matrix are forced towards the diagonal. Clusters emerge in the reordered matrix (figure 2*c*(i)) representing groups of seed voxels with similar patterns of connectivity, which, when mapped back onto the brain, correspond to discrete, spatially contiguous regions situated along the anterior–posterior axis of the medial frontal cortex (figure 2*c*(ii)). The boundary between clusters in the reordered matrix corresponds to a sharp change in connectivity in medial frontal cortex. This border appears close to the coronal plane of the VCA line, supporting the hypothesis that the clusters correspond to SMA and preSMA.

3. FUNCTIONAL/STRUCTURAL RELEVANCE OF DIFFUSION-BASED PARCELLATIONS

In each of the parcellation strategies described above, grey matter regions have been distinguished on the basis of differences in either local or remote white matter architecture. These differences may be interesting in their own right, but the associated hypothesis is that the connectional boundaries which they imply are also of cytoarchitectonic and functional relevance. In this section, we will examine possible strategies for testing this hypothesis, and hence of approaching the related problem of validating the connectivity-based parcellations.

(a) *Structural validation*

The most conclusive method for testing the relationship between connectional and cytoarchitectonic boundaries would be to perform direct comparisons between structural parcellations inferred from *in vivo* diffusion imaging and those derived from *post-mortem* histological staining in the same subject brains. Such comparisons are feasible, but would be challenging, requiring access to the same brains *post-mortem* and *in vivo*. In the absence of these *direct* comparisons, the same question may be addressed indirectly, either by comparing diffusion and histological results from different groups of brains drawn from the same population, or by comparing, in the same brains, diffusion-based parcellations with other known *in vivo* markers of cytoarchitecture.

The first of these approaches relies on a high degree of consistency across the population in connectional, and by inference histological, parcellations. Each of the studies described in §2 tests the population variability in the recovered parcellations and, in each case, demonstrates good reproducibility across a group of subjects.[1] In both of the thalamic studies, where the histo-architecture is well defined, the authors are then able to make comparisons between group average measurements derived from their diffusion-based parcellations, and measurements derived from post-mortem histological atlases. Wiegell *et al.* choose to examine the geometric location of the recovered nuclei within thalamus, demonstrating good correspondence with the centres of mass of nuclei defined by characteristic fibre orientation measured histologically (Niemann *et al.* 2000; figure 3*a*). Johansen-Berg *et al.* (2005) demonstrate consistency between the relative volumes of thalamic regions projecting to each cortical zone and these same volumes estimated on the basis of a cytoarchitectonic atlas of thalamic nuclei (Morel *et al.* 1997).

The ability to test the correspondence between cyto- and connectional architectures in the same brain *in vivo* relies on the existence of reliable alternative *in vivo* markers of cytoarchitecture. Such markers are rare, as in general, cytoarchitectonic boundaries do not align well with gross anatomical landmarks (e.g. Amunts *et al.* 1999). However, in thalamus, recent studies have been able to identify the medio-dorsal and ventral posterior nuclei using specifically designed structural MR protocols. By acquiring such images in the same subjects used for the connectivity-based thalamic parcellation, Sillery *et al.* (in preparation) are able to perform a direct *in vivo* comparison (figure 3*b*).

(b) *Functional relevance*

The relationship between cyto- and connectional architectures explored in §3(a) is principally of interest

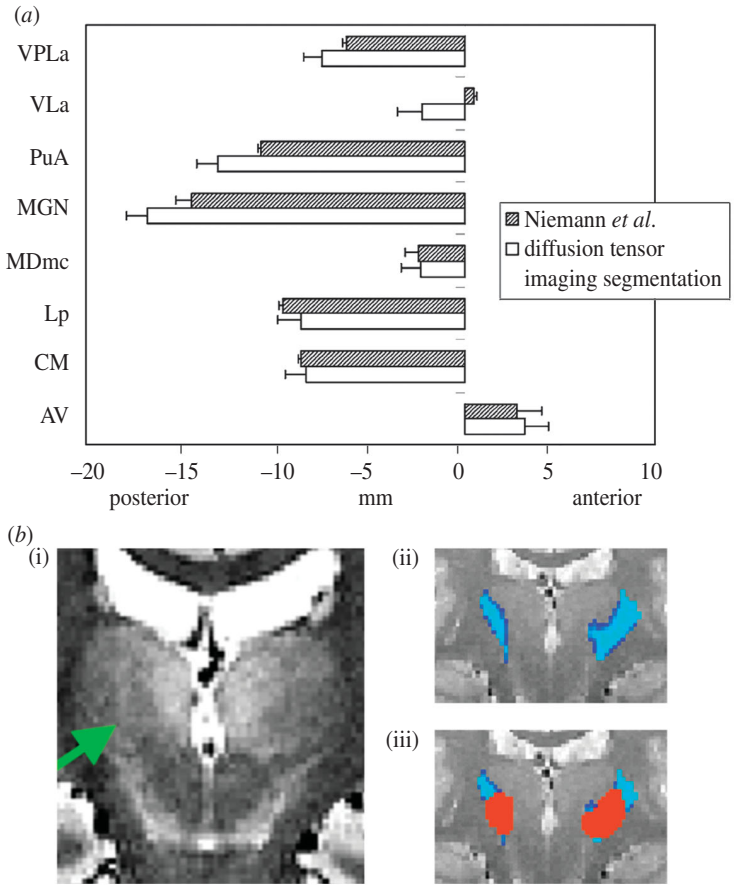


Figure 3. Structural validation of diffusion-based grey matter parcellations: (*a*; taken from Wiegell *et al.* 2003) Comparison between centres of gravity of thalamic nuclei defined on the basis of local diffusion characteristics (white bars), and the same nuclei defined histologically on the basis of local fibre orientation (hashed bars; Niemann *et al.* 2000). Coordinates are along the anterior–posterior axis of the MNI-152 average brain (Evans *et al.* 1992). Similar results are available for the medial–lateral and superior–inferior axes (Wiegell *et al.* 2003). (Figure taken from Wiegell *et al.* (2003).) (*b*; Sillery *et al.* in preparation) Comparison between ventral–posterior nucleus of thalamus defined by remote connectivity to sensory cortices, and by contrast enhanced structural MRI (Sillery *et al.* in preparation). (i) Proton density-weighted MRI shows a hypo-intense region corresponding to the ventral posterior nucleus of thalamus (green arrow). (ii) Region of thalamus defined by 25% chance of connection to sensory cortices. Data from macaque predict that this region will correspond to ventral posterior nucleus. (iii) Manually defined outline of ventral posterior nucleus drawn on (i) and overlaid on (ii). (Figure courtesy of Emma Sillery, FMRIB Centre, University of Oxford.)

because of the known correspondence between cyto- and *functional* architectures. However, the ability to explore connectional architecture *in vivo* allows for comparisons with function to be made explicit. Again, these comparisons may be made indirectly across groups of subjects within a population, or directly in the brains of the same individuals.

In Johansen-Berg *et al.* (2005) we test the hypothesis that connectivity-defined regions in thalamus co-localize with functional activations during tasks expected to involve specific thalamic nuclei and their cortical connectivity targets. Executive and memory tasks are expected to involve the thalamic region projecting to the prefrontal cortex, whereas sensorimotor tasks are predicted to activate thalamic regions projecting to sensory and motor cortices. We compare the locations of connectivity-defined regions grouped across individuals with those of centres of mass of previously reported functional activations in thalamus during executive and sensorimotor tasks (figure 4*a*). Note the spatial dissociation in thalamus between activations associated with the different tasks (figure 4*a*(i)). Centres of activation for the sensorimotor tasks fall within thalamic regions with high group probability of connection to sensorimotor and premotor areas (figure 4*a*(ii)). Similarly, activation centres for the executive and memory tasks fall within thalamic regions of high group probability of connection to prefrontal cortex (figure 4*a*(iii)).

By acquiring functional and diffusion imaging data in the same group of subjects, it is possible to make direct comparisons between connectional and functional borders. Using functional MRI in conjunction with tasks designed to activate SMA and preSMA selectively, we are able to identify the functional boundary between the two regions (figure 4*b*(i); Johansen-Berg *et al.* 2004). The combination of the regions activating during each of the two functional tasks is chosen as a seed region in which to search for a connectional dissociation in the diffusion data. As before, probabilistic tractography is seeded from every voxel in this region; the connectivity

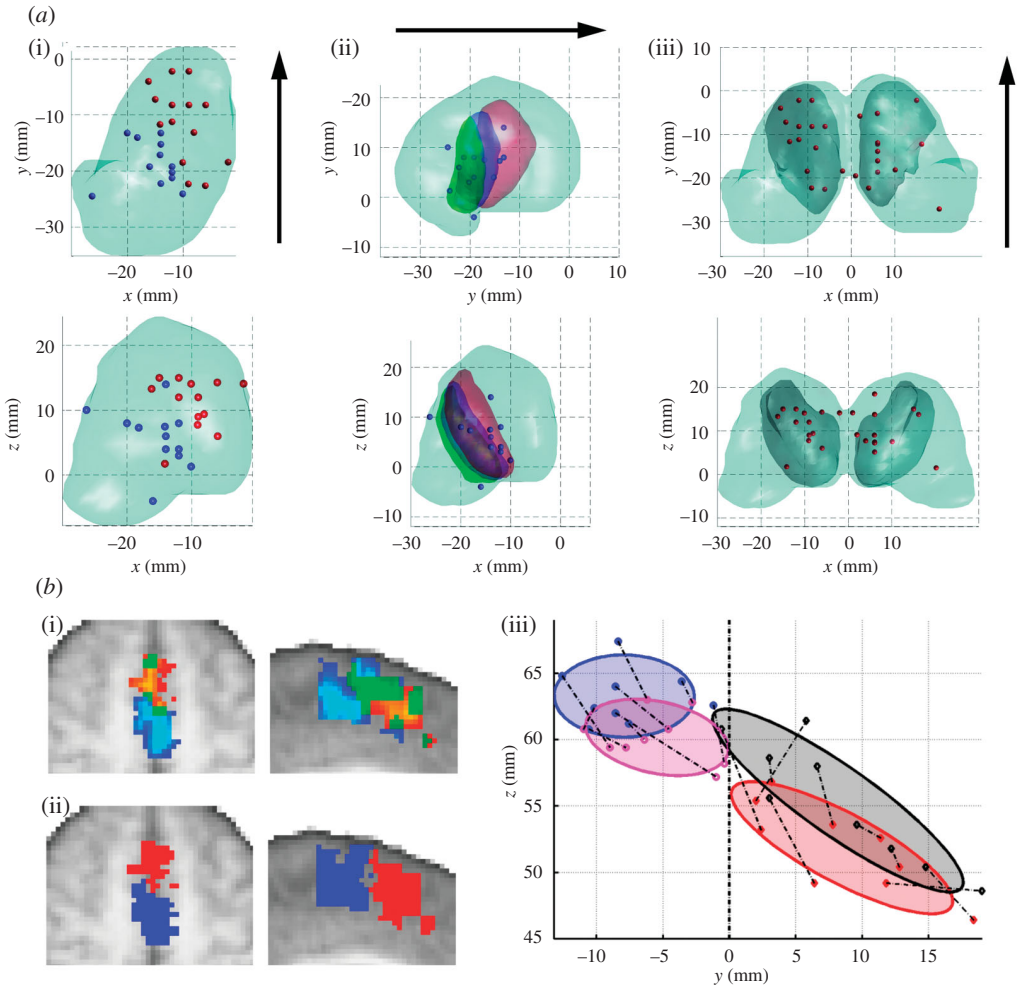


Figure 4. Functional relevance of diffusion-based parcellations. (*a*) Functional relevance of connectivity-based parcellation of thalamus. In each figure, the pale grey surface represents the thalamus; the black arrow represents a posterior–anterior orientation; spheres represent reported centres of functional activation in executive (red) and sensorimotor (blue) tasks; inner semi-transparent surfaces represent thalamic regions in which at least four out of 11 subjects had a probability of 25% of connection to a particular cortical zone. (i) Spatial dissociation between reported thalamic centres of functional activation in sensorimotor and executive tasks. (ii) Thalamic centres of functional activation in sensorimotor tasks aligned well with regions defined by connectivity to sensory (green), primary motor (blue) and premotor (red) cortices. (iii) Thalamic centres of functional activation in executive tasks aligned well with regions defined by connectivity to prefrontal cortex (dark grey). (Figure taken from Johansen-Berg *et al.* 2005.) (*b*) Functional relevance of connectional dissociation in medial area 6. (i) Simple motor and cognitive tasks selectively activate SMA (dark to light blue) and preSMA (red to yellow). (ii) Seed voxels which were activated by either cognitive or motor tasks were submitted to connectivity-based parcellation (see figure 2 and main text). A connectional dissociation was found which matched the functional dissociation in (i). (iii) Correspondence between centres of gravity of functionally defined (magenta and black) and connectionally defined (blue and red) SMA and preSMA in nine subjects. Coordinates are in MNI space (Evans *et al.* 1992) on the mid-sagittal plane. (Figure based on Johansen-Berg *et al.* (2004), ©2004 by the National Academy of Sciences.)

profile correlation matrix is computed and submitted to spectral reordering; and clusters emerge in the reordered matrix. When mapped back onto the brain, the regions defined using connectivity information alone bear close resemblance to the functionally defined SMA and preSMA (figure 4*b*).

4. CONCLUSIONS

The connectional architecture of a brain region constrains the region's function. Magnetic resonance diffusion imaging offers information about this architecture that is unique among non-invasive measurement techniques. Using this information, it is possible to identify regions in cortical and subcortical grey matter that can be dissociated by features of their connectivity, and demonstrate that the connectional boundaries that these dissociations imply have relevance in terms of both cellular and functional architecture.

In addition to the studies reviewed in this article, recent and ongoing studies have successfully applied similar techniques to subdivide different brain areas. For example, in Lehericy *et al.* (2004), using remote cortical connectivity patterns, the authors are able to subdivide the striatum into regions belonging to distinct cortico-striatal circuits. In Sillery *et al.* (2004), differences in connectivity patterns reveal a dissociation between the internal and external globus pallidus. The authors demonstrate a correspondence between boundaries identified by changes in connectivity and those identified manually on a high-resolution proton-density-weighted MR scan.

A general relationship between boundaries in connectional architecture visible to diffusion imaging and borders between cytoarchitectonically and functionally distinct grey matter regions would be of great significance to future neuroimaging studies. Such a relationship would allow future functional imaging results to be interpreted in the context of local cytoarchitectonic borders. Functional data could be compared directly across studies with reference to the cytoarchitectonic region of the activation site. Functionally relevant boundaries could be preserved when aligning data from different subjects, allowing, for example, for more accurate measurements of variability in functional responses across a population. The ability to infer cytoarchitectonic boundaries *in vivo* would also have significance for studies of brain structure. Boundary locations, or regional volumes, could be compared across subject groups or brain hemispheres. Lesion locations could be estimated accurately with respect to the cytoarchitecture.

However, although the strategies reviewed in this article have allowed for initial demonstrations of the potential of diffusion data for inferring structural and functional parcellations, they are not yet in a position to provide generally applicable tools. Any technique designed to group data into dissociated clusters relies on the ability to make hard decisions about, for example, the number of clusters to choose and the membership of each cluster. To date, diffusion-based parcellation studies have relied on manual setting of these parameters—Weigell *et al.* (2003) chose to search for 14 thalamic nuclei. Johansen-Berg *et al.* (2004) identified clusters by eye in the reordered correlation matrices. Before these techniques can be generally applied to blind structural parcellation in grey matter, statistical techniques should be developed to provide an automated framework in which to make these decisions. This problem is closely related to problems faced by scientists attempting to infer similar parcellations from classical histology. Robust methods for making decisions about the existence and location of boundaries in cellular architecture are the subject of ongoing research (e.g. Schleicher *et al.* 2000).

The methods that we have described have all relied on explicit models of local diffusion in biological tissue. These models make simplifying assumptions, such as the existence of only a single characteristic fibre orientation within any voxel, which may preclude them from identifying boundaries in regions of the brain where these assumptions break down. Recent research has allowed the representation of local diffusion information within a voxel without the need for these restrictive models (Alexander *et al.* 2002; Tuch *et al.* 2003). This new technology will only improve the sensitivity and accuracy of all diffusion-based connectivity studies.

The spatial resolution of the parcellations is also limited by current technology. A set of diffusion-weighted images appropriate for tractography might take 40 min to acquire, and have spatial resolution of only $2\times2\times2\ \mathrm{mm}^3$. As MR scanner technology improves, the spatial resolution of the images will increase, allowing for finer anatomical parcellations.

Despite these limitations, we have shown that diffusion-based parcellations, carried out in controlled circumstances in which functional or cytoarchitectonic boundaries are available, have identified connectional borders which align well with the functional or structural data. In the cases where the inferred parcellations depend on the accuracy of the underlying diffusion tractography, the functional/structural validation of these parcellations may prove a first step towards validating the tractography process. Eventually, the validity of diffusion-based connectivity studies should be established by direct comparison with invasive tracer studies in non-human primates. However, at present, the voxel resolution available to diffusion studies (of the order of $1\ \mathrm{mm}^3$) is such that only the largest fibre pathways are visible in a macaque brain (Parker *et al.* 2002). The demonstration of good correspondence between connectional, functional and cytoarchitectures inferred from diffusion imaging, functional imaging and histolology, respectively, not only promises to provide powerful tools for studying brain structure and function *in vivo*, but lends weight to the argument that diffusion imaging is providing measures relating to anatomical brain circuitry.

ENDNOTE

[1]Note that group reproducibility of the thalamic parcellation described in Behrens *et al.* (2003*a*) is examined in detail in Johansen-Berg *et al.* (2004).

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