Using Diffusion Imaging to Study Human Connectional Anatomy

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Abstract
Diffusion imaging can be used to estimate the routes taken by fiber pathways connecting different regions of the living brain. This approach has already supplied novel insights into in vivo human brain anatomy. For example, by detecting where connection patterns change, one can define anatomical borders between cortical regions or subcortical nuclei in the living human brain for the first time. Because diffusion tractography is a relatively new technique, however, it is important to assess its validity critically. We discuss the degree to which diffusion tractography meets the requirements of a technique to assess structural connectivity and how its results compare to those from the gold-standard tract tracing methods in nonhuman animals. We conclude that although tractography offers novel opportunities it also raises significant challenges to be addressed by further validation studies to define precisely the limitations and scope of this exciting new technique.
INTRODUCTION

Anatomical connections in the brain are of interest to neuroscientists because connectivity patterns define functional networks. The inputs to a brain region determine the information available to it, whereas its outputs dictate the influence that that brain region can have over other areas. Therefore, simply by knowing the pattern of inputs and outputs of a brain region we can begin to make inferences about its likely functional specialization. Consider the lateral intraparietal area (LIP) in the monkey; anatomical studies tell us that this region receives strong inputs from the dorsal visual stream and has connections with brain regions such as the frontal eye fields and the superior colliculus, which are involved in the control of eye movement (Cavada & Goldman-Rakic 1989a,b; Lewis & Van Essen 2000). This information alone supports the notion that the region may play a visuospatial role and contribute to the control of eye movements, an idea supported by functional studies (Andersen & Buneo 2002, Bisley & Goldberg 2003). This review discusses the possibilities and limitations offered by diffusion imaging, a noninvasive brain imaging approach, in studying connectional anatomy in the human brain.

There has been a long history of visualizing white-matter pathways in the brain (Schmahmann & Pandya 2006). In the seventeenth century, Raymond de Vieussens developed methods for boiling the brain in oil to strip back the outer brain tissue and reveal the underlying fiber structure. Early dissection methods followed, then Joseph Jules Dejerine, at the end of the nineteenth century, used a myelin stain on serial brain sections to visualize the routes of association pathways through the brain. All these approaches relied on passively visualizing brain pathways. Later techniques, such as those of Marchi or Nauta, applied selective lesions to areas of interest and then followed the degeneration of interconnected axons by using axonal stains.

Contemporary methods for tract tracing exploit the active transport mechanisms of the cell. A tracer substance, which can be, for example, a virus, a fluorescent dye, or an enzyme, is injected into an area of interest in a living animal. For retrograde tracing, the substance is taken up by axon terminals and transported back to the cell body. For anterograde tracing, the tracer is taken up by the cell body and transported along the axon to the terminals. Following a survival period to allow active transport to occur, the animal is sacrificed and the brain fixed, sectioned, and processed to allow for tracer visualization. Such techniques have advanced enormously in sophistication, and current approaches allow, for example, application of multiple tracers to label many paths in a single experiment (Morecraft et al. 2009).

Tracer techniques have provided a wealth of data in various animals, particularly nonhuman primates, for which we have an array of complementary information concerning the function of brain regions from electrophysiological and lesion studies.

A principle that has emerged from these data is that each brain region has a unique
connectivity fingerprint. For example, studies of the macaque monkey have shown that although it is not unusual for brain areas to share some common connections with other regions, their overall connection patterns differ from one another. For example, Passingham and colleagues (2002) considered the published evidence for anatomical connections of the prefrontal cortex and demonstrated that areas with different functional roles also had unique patterns of anatomical connections.

Although powerful, tracer techniques are obviously not applicable to the study of the human brain. Yet studies of anatomical connections in human subjects are critical if we wish to test for breakdown of connectivity in disease; to investigate the anatomy of regions for which there is no clear homolog in nonhuman animals; or to study structure, function, and behavior in the same individuals. Recent developments in noninvasive brain-imaging techniques provide us with new opportunities for investigating connectational anatomy in the human brain.

In diffusion-weighted magnetic resonance imaging (DWI), we sensitize our signal to the self-diffusion of water molecules. This is a useful property to measure because we know that in tissue with directional structure, such as brain white matter, water diffusion is directionally dependent. In a coherent fiber bundle, diffusion is less restricted along the fiber axis than across it, and hence more diffusion will be measured along the fiber axis over a given time (Figure 1a). In a DWI experiment, we acquire multiple brain images, and in each image we sensitize our signal to diffusion along a different direction. In this way, we build up multiple measurements for each brain voxel (three-dimensional pixel). We can fit a mathematical model to these measurements at each voxel. The most commonly used model is the diffusion tensor model, where diffusion at each voxel is described by an ellipsoid, or tensor (Figure 1b). The tensor is fully characterized by its three orthogonal eigenvectors and their associated lengths, or eigenvalues ($\lambda_1$, $\lambda_2$, $\lambda_3$). The shape of the ellipsoid contains information

![Figure 1](https://www.annualreviews.org/doi/10.1146/annurev.neuro.32.060408.134831)

**Figure 1**

Modeling diffusion. (a) Schematic to illustrate diffusion of a water molecule (black line) within a fiber bundle. Diffusion is less restricted along the axis of the fiber bundle than across it. (b) The diffusion tensor model. Water diffusion at each voxel is modeled by a tensor, characterized by its three principal eigenvectors and their associated eigenvalues ($\lambda_1$, $\lambda_2$, $\lambda_3$). (c) Fractional anisotropy (FA) is calculated from the eigenvalues of the tensor fit and ranges between zero and one. FA reflects the shape of the tensor, with more spherical tensors having lower FA values as shown. (d) Simple, streamlining tractography proceeds by tracing a line through the tensor field, following the principal diffusion direction. The schematic shows a grid of voxels; the grayscale reflects FA [ranging from zero (black) to one (white)]. The corresponding tensors are illustrated by the ellipses shown at each voxel. (e) Tractography allows for beautiful reconstructions of major fiber bundles, such as the example of the corpus callosum, shown here. Figure (d) courtesy of D. Jones, based on data from Catani et al. 2002.
Resonance imaging

Magnetic anisotropy

FA: fractional imaging

Weighted tensor DWI:

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Another critical parameter estimated in the diffusion tensor model is the principal diffusion direction, the direction of the long axis of the tensor. Within a coherent fiber bundle, this direction corresponds to the orientation of the underlying pathway. This correspondence is the basis of diffusion tractography, in which directional estimates are followed to reconstruct estimates of the path of fiber bundles (Jones et al. 1999, Mori et al. 1999) (Figure 1d). Such approaches have allowed researchers to produce beautiful in vivo dissections of major fiber bundles in the human brain (Figure 1e) (Catani et al. 2002). Although this approach results in dramatic images, it is important to remember that diffusion tractography does not trace anatomical connections in the way that injection of an actively transported substance does.

First, it is important to clarify what is meant by connectivity in this context. Although we may use diffusion MRI to estimate the likelihood of a pathway existing between two brain areas, it is not possible to test whether synapses are formed between the incoming axons from one brain area and the dendrites in another brain area. Furthermore, it is important to make a distinction between measures of structural and functional connectivity. A number of functional MRI (fMRI)-based techniques correlate activity measures across two or more areas and examine how such correlations change, sometimes from moment to moment, when participants are engaged in a cognitive process (Friston 2005). Information about patterns of functional connectivity can, however, sometimes suggest inferences about structural connectivity (Cohen et al. 2008); areas with strongly correlated activity are more likely to be anatomically connected. Although functional connectivity is presumed to depend on structural connectivity, investigators assume that basic aspects of structural connectivity, dependent on the presence of axons running between two
brain areas, remain constant or change only over longer time periods during the course of learning or development.

**TO WHAT EXTENT DOES DIFFUSION TRACTOGRAPHY MEET THE CHALLENGES FOR A STRUCTURAL CONNECTIVITY METHOD?**

The remainder of this article takes a critical view of the degree to which tractography meets the challenges for a method proposed to study structural connectivity. We consider the following criteria: the ability to trace distributed cortico-cortical networks, perform fine-grained topographic mapping, provide quantitative information, determine tract polarity, and define the presence or absence of a connection.

**Tracing Distributed Cortico-Cortical Networks**

The first challenge for a structural connectivity method is that it should allow us to study the cortico-cortical networks that were discussed at the beginning of this review. If a major motivation for studying connectional anatomy is to inform our investigations of functional networks, then the ability to study cortico-cortical networks is a basic requirement. It is, however, challenging for some tractography algorithms to trace pathways to their gray-matter destinations. So-called streamlining techniques, which simply follow directional estimates derived from a tensor model as described above, can easily reconstruct major fiber bundles but cannot continue tracing when there is uncertainty over fiber direction. As fibers approach gray matter (or pass through regions of fiber crossing), anisotropy reduces, reflecting the divergence of fibers. A simple line-following approach cannot handle this scenario and stops tracing when FA falls below a given threshold (typically 0.2). However, a class of alternative tractography approaches, which operate within a probabilistic framework, allow for tracking even in the presence of uncertainty (Behrens et al. 2003b, Parker & Alexander 2005). With such approaches, a probabilistic model, rather than a diffusion tensor model, is fit to diffusion measurements. So, rather than providing a point estimate of the principal diffusion direction, such models also estimate a probability distribution on this direction at each voxel, with broad probability distributions reflecting increased uncertainty. At the tractography stage, rather than drawing a single line through direction estimates, sampling techniques are used to draw thousands of streamlines through this probability field to build up a connectivity distribution, where the density of streamline samples reflects the probability of interconnection with the seed voxel. There are many benefits from operating within a probabilistic framework. First, tracking can continue in the presence of uncertainty and thus paths can be traced to their gray-matter targets. Second, these techniques provide a quantitative measure of the probability of a pathway being traced between two points; such measures can be used to perform quantitative comparisons between groups of subjects, although we should be cautious about how to interpret such values in biological terms. Finally, probabilistic models can be extended to fit multiple fiber populations (Behrens et al. 2007, Parker & Alexander 2005, Tuch 2004), a critical feature if paths are to be traced through regions of fiber complexity.

Having established that tractography methods are available for tracing cortico-cortical networks, we now review two specific examples. First, consider the posterior parietal cortex. This is a region that we know a great deal about in the macaque monkey, but correspondences with human parietal cortex are not always clear (Glover 2004, Husain & Nachev 2007). The principal division within the parietal cortex is between the superior parietal lobule (SPL) and the inferior parietal lobule (IPL). The two lobules are divided by the intraparietal sulcus (IPS). It has frequently been argued that human IPL is unique and only distantly related to IPL in other primates. This claim is based partly on the fact that Brodmann used the

- **IPL**: inferior parietal lobule
- **IPS**: intraparietal sulcus
numbers 39 and 40 to refer to human IPL but did not use these labels when describing the monkey brain (Brodmann 1909) (Figure 2a). Other anatomists, however, have emphasized similarities between the IPLs of human and monkeys (Eidelberg & Galaburda 1984, Von Bonin & Bailey 1947). We wished to test whether tractography could help test hypotheses about correspondences between species in this brain region.

Electrophysiological studies have shown that IPS regions have distinct functional specializations; neurons in lateral IPS (LIP) respond during eye movements or shifts in attention, whereas neurons in medial (MIP) and anterior (AIP) parts of IPS respond when monkeys make arm movements (Andersen & Buneo 2002, Bisley & Goldberg 2003, Sakata et al. 1997). These functional distinctions are mirrored by differences in anatomical connections; each subregion forms part of a distinct anatomical circuit. Based on previous tracer studies in macaque monkeys, we identified three connections that distinguish LIP, AIP, and area 7a, which lie on the convexity of the IPL. Dense connections exist between superior colliculus and LIP (Clower et al. 2001, Lynch et al. 1985), parahippocampal gyrus and area 7a (Seltzer & Pandya 1984), and ventral premotor cortex and AIP (Matelli et al. 1986). Importantly, the pathway taken by each of these projections is known to be different (Gaymard et al. 2003, Petrides & Pandya 2006, Seltzer & Pandya 1984), so a priori reasons support the thinking that they might be distinguished by diffusion tractography.

We used tractography in human subjects to test whether we could identify parietal areas with corresponding connection patterns (Rushworth et al. 2006). We found a region in and superior to the IPS that connected with superior colliculus, a region in the angular gyrus in the posterior IPL that connected with the parahippocampal gyrus, and an anterior IPS/IPL region that connected with ventral premotor cortex via the superior longitudinal fascicle (Figure 2b). The connection patterns and relative spatial positions (Figure 2c) of the posterior and anterior IPL regions are similar to those of the monkey, suggesting that human IPL shares basic features of its organization with monkeys. However, the study also highlighted differences between the species. Whereas in the monkey brain the superior colliculus is connected exclusively with area LIP on the lateral bank of the IPS, in the human brain there was strong evidence for superior collicular connectivity with the medial bank of IPS (Figure 2b,c). fMRI studies have also suggested that human medial IPS has functional properties reminiscent of monkey LIP (Sakata et al. 2001, Silver et al. 2005). Such findings suggest a relative expansion of LIP in humans.

Another region for which questions remain about homologies between human and monkey anatomy is the lateral premotor cortex. In the macaque, this region is divided into several fields, with an important division between dorsal (F2) and ventral premotor regions (F4 and F5) (Barbas & Pandya 1987, Matelli et al. 1985) (Figure 3a). Whereas dorsal premotor areas...
\[ Z = 63 \]

\[ X = 55 \]

\[ Y = 83 \]

\[ Z = 34 \]

\[ X = 40 \]
(PMd) are involved in learned, nonstandard stimulus response mappings (Wise et al. 1996), more ventral regions (PMv) play a role in visually guided reaching and grasping (Jeannerod et al. 1995). In line with their different functional specialization, PMd and PMv have distinct connection patterns that can be well characterized by considering connections with prefrontal and parietal cortex (Geyer et al. 2000). Whereas PMd is interconnected with superior parietal and dorsal prefrontal and cingulate areas, PMv has strongest connections with anterior and inferior parietal areas and with ventral prefrontal regions. Human premotor cortex (the lateral part of Brodmann’s Area 6) is also divided into cytoarchitectonically (Vogt & Vogt 1919) and functionally (Amiez et al. 2006, Schubotz & von Cramon 2001, Toni et al. 2001) distinct dorsal and ventral subregions. In vivo, however, the border between these subregions is unclear (Figure 3a).

We used diffusion tractography to test two hypotheses about human premotor cortex (Tomassini et al. 2007). First, the border between human PMd and PMv could be defined based on differences in their anatomical connections, and second, the differences in connectivity patterns of these two regions would match predictions from data in macaques. Using probabilistic tractography to define connectivity profiles from each voxel within the human premotor cortex, we clustered voxels based on similarities in their connection patterns using previously described techniques (Anwander et al. 2007, Johansen-Berg et al. 2004). We defined two clusters with distinct connectivity patterns, which we propose correspond to human PMd and PMv (Figure 3b). The border between these regions was located in a consistent location with respect to local sulcal landmarks, about halfway between the superior and inferior frontal sulci (Figure 3c), consistent with previous suggestions based on macaque anatomy (Rizzolatti et al. 1998). When the connectivity patterns of these two regions with a number of predefined parietal and prefrontal regions were quantified, we found that the connectivity fingerprints of the DWI-defined PMd and PMv regions matched those predicted for PMd and PMv from studies in nonhuman primates (Figure 3d).

The case studies of the parietal and premotor cortex demonstrate that probabilistic tractography can be used to map cortico-cortical connections in the human brain. Results obtained from such studies partly reinforce

**Figure 3**

Parcellating human premotor cortex based on anatomical connectivity. (a) Both human (left) and macaque (right) premotor cortex can be divided into cytoarchitectonically distinct subregions, but determining these boundaries in vivo is challenging (Brains are not shown to scale). (b) Connectivity-based parcellation of human lateral premotor cortex defines two regions with distinct connection patterns: putative dorsal (blue) and ventral (red) premotor cortex, shown here overlaid onto a coronal section for a single subject. The matrix shown is the k-means clustered connectivity cross-correlation matrix, in which two clusters with distinct connection patterns are clearly discernable. Rows and columns in the matrix correspond to voxels within the premotor cortex. Correlations (in connectivity patterns) across voxels are high within a cluster and low between clusters. (c) Projection of the PMv/PMd border onto the brain surface indicates that the border is located in a relatively consistent location with respect to local sulcal landmarks, between the superior (SF) and inferior frontal (IF) sulci, close to the horizontal extension of the inferior frontal sulcus (HE/IF). (d) Connectivity fingerprints for PMd (blue) and PMv (red) illustrating strength of connection with parietal and prefrontal subregions. (e) The results of a meta-analysis of functional studies (based on Mayka et al. 2006) are shown by the contour lines in the background. Black contour lines indicate activations in PMd, and blue contour lines indicate activations in PMv. Overlaid on this are the connectivity-based regions corresponding to PMd (blue) and PMv (red), illustrating the good correspondence between structural and functional information. Figure 3a is based on Rizzolatti et al. (1998) with permission. Figures b-e are based on data from Tomassini et al. (2007).
previous findings from nonhuman primates but can also highlight potential differences between species.

**Fine-Grained Mapping of Anatomical Connections**

A second challenge requiring a structural connectivity method is to perform fine-grained mapping of anatomical connections. With tracer studies in nonhuman animals, mapping is performed at a microstructural level, and exquisite spatial detail can be obtained. With electron microscopy, it is possible to visualize individual components on synaptic terminals on injected cells (Lacey et al. 2007). Even using light microscopy, researchers can determine not only the precise cortical area that is innervated by a particular connection, but also which specific layer. Tracking pathways at the level of individual axons, or even cortical layers, is beyond the scope of diffusion tractography using currently available techniques that typically provide images with a resolution in the order of a few cubic millimeters. So how far can we push the effective resolution of tractography to obtain fine-grained spatial mapping? A good test case is provided by the thalamus, a deep gray-matter structure that is divided into cytoarchitectonically distinct nuclei. The thalamus is sometimes thought of as just a relay station for incoming sensory information to the cortex, but its component nuclei are connected with most cortical regions, including motor regions, and it is clear that its neurons are actively engaged in processing the information that they receive (Wanderlich et al. 2005). Divisions between nuclei can be readily visualized using histological techniques, but none of this intrinsic structure is visible on a conventional T1-weighted MRI scan. Yet we know that each thalamic nucleus is concerned with a different type of information and connects with different parts of the cerebral cortex. Is it possible to exploit this knowledge to obtain a finer-grained mapping of thalamic anatomy than can be achieved using conventional MRI? We previously showed that diffusion tractography can be used to trace pathways from each voxel within the human thalamus and then classify those voxels according to the cortical area with which they have the highest probability of connection (Behrens et al. 2003a) (Figure 4a). This procedure defines a striking and reproducible organization within the thalamus, identifying discrete clusters that are proposed to correspond to nuclei or nuclear groups (Johansen-Berg et al. 2005). Although this analysis provides a window on in vivo anatomy that was not previously available, it still reflects a rather crude description of thalamocortical relationships. We know from studies in monkeys that there is a topographic mapping of connections between thalamus and cortex (Ray & Price 1993, Shipp 2001). For example, regions within prefrontal cortex (PFC) are organized within distinct, distributed anatomical circuits. Three such circuits are centered on the lateral orbitofrontal cortex, medial frontal and cingulate cortices, and lateral prefrontal cortex. Some of the first anatomical evidence for these networks came from tracer studies showing that these regions have distinct connectivity patterns with the mediodorsal nucleus of the thalamus (Ray & Price 1993). We used diffusion tractography on diffusion tensor imaging (DTI) data in humans and macaque monkeys, to test for the existence of this topography (Klein et al. 2007a). Tractography results in the macaque broadly echoed results described previously using tracers, whereas results in the human demonstrated that a similar topography can be detected in the human brain (Figure 4b).

**Quantitative Information on Connection Strength**

A structural connectivity method ideally should provide information on the strength of anatomical connections to allow us to test hypotheses about differences in connection patterns between brain regions, individuals, time points, or species. Making inferences about connection strength is challenging even for gold-standard tract tracing techniques; it is difficult, for example, to establish whether equivalent amounts of
Testing thalamo-cortical topography with tractography. (a) (i) On conventional MRI, it is not possible to visualize the intrinsic structure of the thalamus, yet we know from histology (ii) that it consists of cytoarchitectonically distinct nuclei. We identified cortical target regions (iii) and then classified thalamic voxels according to the cortical region with which they had the highest probability of connection (iv). This results in a clear, reproducible clustering that is proposed to identify nuclei or nuclear groups. (b) (i) Tracer studies in macaque suggest fine-grained topographic mapping between the mediodorsal nucleus (MD) and prefrontal cortex (PFC). Schematic is of a coronal section through MD, based on results from Ray & Price (1993), modified so that regions with distinct connectivity are color coded (magenta, anterior cingulate cortex; yellow, lateral orbitofrontal cortex; green, dorsolateral PFC; blue, lateral PFC). (ii) Shows equivalent topography based on diffusion tractography in macaque. Coronal slices through macaque MD are shown and voxels are color coded (as per i) according to their probability of connection with subregions of PFC. The topography apparent from the tractography data is broadly similar to that present in the tracer data. Figure a is based on data from Behrens et al. (2003a) and Johansen-Berg et al. (2005). Figure b is based on data from Klein et al. (2007a).
myelin thickness, packing density) and a number of potential confounding factors (features of unrelated, crossing fiber populations, tract geometry) will also contribute to these diffusion measures. Likewise, tractography algorithms can provide quantitative measures such as the number of streamlines connecting two points or the strength or probability of a connection, but again, in addition to the true strength of the underlying pathways, other features such as tract length and data quality will influence these measures. Therefore, any tractography-derived measure should be interpreted with care.

Nevertheless, investigators have shown that diffusion measures have functional and clinical relevance. Fractional anisotropy varies with development (Barnea-Goraly et al. 2005, Giorgio et al. 2008), aging (Pfefferbaum et al. 2000, Salat et al. 2005), and disease (Cercignani et al. 2001, Ciccarelli et al. 2008). Even within a healthy, homogenous adult population, interindividual variation in behavioral performance on a range of motor and cognitive tasks correlates with localized variation in fractional anisotropy in specific, task-relevant pathways (Johansen-Berg et al. 2007, Tuch et al. 2005). Furthermore, associations have been found between diffusion measures and measures of functional connectivity such as those derived from functional MRI (Putnam et al. 2008) or transcranial magnetic stimulation (Boorman et al. 2007, Wahl et al. 2007). Together, these studies suggest that variation in measures such as fractional anisotropy is meaningful and functionally significant.

**Accurate Definition of the Presence or Absence of a Connection**

Ideally, a structural connectivity method should provide us with definite evidence on the existence of a particular tract. However, like most techniques, tractography is susceptible to false positives and false negatives. The inability to trace a pathway should not be taken, in itself, as strong evidence that the pathway does not exist. Similarly, if an unexpected pathway is traced, then the interpretation that a new route has been discovered should be made only with extreme care.

A few examples of unusual pathways being traced in rare subjects with early brain damage (Bridge et al. 2008, Leh et al. 2006) or blindness (Shimony et al. 2005) suggest that extreme reorganization (or change in the relative strength) of brain wiring may be detected in such cases. Even in a healthy population, there is interindividual variation in the ease with which some pathways can be traced, and in some cases, this variability has functional relevance. For example, in a study of language pathways in the human brain, Catani and colleagues (2007) report that in ~60% of the population, it is not possible to trace the direct portion of the arcuate fasciculus in the right hemisphere. This pattern of extreme lateralization, which was particularly
common in males, was associated with poorer performance on verbal learning tasks (Catani et al. 2007). Does this finding reflect striking anatomical variation within healthy adults, such that a major white-matter pathway is absent in one hemisphere in more than half the population? Arguably, a more plausible explanation is that anatomical variation (in, for example, fiber density, myelination, geometry, etc.) results in the path being easier to trace using tractography in some subjects compared with others. This explanation does not render the result uninteresting; it is highly likely that the difference in tractography performance reflects meaningful anatomical variation, but we should be cautious before interpreting such results as providing evidence for the absence of a tract.

The challenge in differentiating between an exciting new discovery and a false positive arises in part from the limited number of validation studies that exist for tractography. As mentioned above, a number of studies have attempted to clarify the biological basis for local diffusion measures such as fractional anisotropy by using model systems in which factors such as myelin, axon density, or diameter are systematically varied. Comparable validation studies are difficult to achieve with tractography. The final section of this article reviews progress in validation studies.

THE CHALLENGE OF VALIDATION

To test validity directly, results from tractography should be compared with some known ground truth. This can be done using phantoms, in which an artificial fiber architecture is known (Perrin et al. 2005), but it is extremely difficult to produce biologically realistic phantoms in this context. Alternatively, tractography results can be compared with results from gold-standard invasive tract tracing methods. Croxson and colleagues (2005) used diffusion weighted imaging to estimate the connections of several white-matter pathways and subcortical nuclei, including the uncinate fascicle, extreme capsule, cingulum bundle, branches of the superior longitudinal fascicle, hippocampus, amygdala, and dorsal and ventral striata, with frontal cortex in both macaques and human subjects (Croxson et al. 2005). It was therefore possible to compare the macaque tractography data with the known frontal connections of these regions that have been established in tracer studies (Figure 5a) and show that there were broad similarities in the connection patterns detected across techniques (Figure 5b). As a second stage, the study then went on to compare DTI tractography–defined connections in macaques and humans. Although once again broad similarities between connection patterns were evident in the two species, there were also informative differences. For example, as was expected from tract tracing, the DTI-tractography data in the macaque showed that the third branch of the superior longitudinal fascicle was strongly interconnected with the ventral premotor cortex but only relatively weakly interconnected with the ventral prefrontal cortex (Figure 5b). In the human brain, however, the same fascicle connected with the posterior part of Broca’s area (pars opercularis), a language region that is often included within the prefrontal cortex (Figure 5c). However, there was little evidence that other fascicles that strongly interconnect with the prefrontal cortex in the macaque, such as the extreme capsule, were interconnected with the human pars opercularis (Figure 5c). The pars opercularis lies only just anterior to the ventral premotor cortex, and the pattern of DTI-tractography results suggest that this region has important similarities with premotor cortex, as opposed to prefrontal cortex, in other primate species (Figure 5d). The tractography results, together with recent neuroimaging, neurophysiological, and neuroanatomical studies, concur that these parts of the language system likely must be understood within the context of the motor system (Petrides et al. 2005).

It is important to know the trajectory taken by DTI tractography–defined fiber bundles to compare them with the known trajectories.
Figure 5
Anatomical connectivity of prefrontal cortex in macaque and human. (a) Tracer studies have identified the location of the two major fiber bundles connecting posterior brain areas with the ventral frontal cortex. There is, however, a difference in the degree to which these fascicles are interconnected with premotor and the prefrontal divisions of the frontal lobe. The extreme capsule (EC, left) connects the temporal lobe to the ventral prefrontal cortex, whereas the superior longitudinal fascicle (SLF), particularly its third branch (SLFIII), connects the parietal cortex with ventral premotor cortex (PMv) (based on Schmahmann et al. 2007). (b) Tractography studies confirm that the macaque SLF (blue) is more strongly interconnected with PMv, whereas EC (green) is more strongly connected with ventral prefrontal cortex (PFv). (c) Diffusion tractography can also be used to estimate the connections of the ventral frontal lobe of the human brain. In the left hemisphere, an important part of the ventral frontal cortex is called Broca's area, a region involved in language and often thought of as a prefrontal brain region. This region consists of an anterior part (aBA, including the pars triangularis, which corresponds largely to Brodmann area 45) and a posterior part (pBA, the pars opercularis, which is composed largely of Brodmann area 44). When the connection patterns of these regions with the EC and SLFIII are compared, it is evident that anterior Broca's area connections (cyan) are reminiscent of the macaque PFv, whereas posterior Broca's area connections (magenta) are reminiscent of the macaque PMv. (d) The results suggest that anterior Broca's area has similarities with the prefrontal cortex of the macaque and that it is particularly interconnected with the temporal lobe. This finding is consistent with its role in the semantic aspects of language. By contrast, it may be more appropriate to view posterior Broca's area within the context of the premotor system; like the premotor cortex, it is strongly interconnected with the parietal cortex. It may be this part of Broca's area that is particularly important during the motor production of speech. Figures b, c, and d are based on data from Croxson et al. (2005).
to autoradiographic tract tracing findings in the same species (Schmahmann et al. 2007).

An even greater challenge is to compare such findings directly in the same individual animals. One ambitious study in three minipig brains compared postmortem multifiber probabilistic tractography findings to results obtained from two different injected tracers: the histochemically detectable biotinylated dextran amine (BDA) and manganese, which results in enhancement on MRI (Dyrby et al. 2007). After injection into motor, somatosensory, or prefrontal cortex, both tracers tracked corticocortical, corticothalamic, and corticofugal pathways. In general, agreement between results from tractography and tracers was good (overlaps were typically 70%-90%), although some tracts detected using tracers could not be tracked with diffusion data. For example, prefrontal and motor corticothalamic tracts were not consistently traced with tractography, reflecting the finding that tractography pathways did not tend to terminate in thalamus but rather continued past this structure. This difficulty in terminating in intermediate gray-matter structures is probably due to the high anisotropy of the continuing white-matter pathways relative to the low anisotropy of the white/gray border at the entry point to the thalamus. Higher spatial resolution and more complex fiber modeling could improve performance in these situations where minor or branching pathways are missed. Furthermore, some potentially spurious pathways around the superior corona radiata were consistently tracked using tractography but were absent with both tracer methods, consistent with the observation that false positives are often detected in regions of high fiber complexity (Jones et al. 2005, Pierpaoli et al. 2001). Consistent with the limited agreement among techniques observed in this study, a study performing streamlining tractography and histology in a single macaque monkey also indicated good qualitative agreement between pathways but showed significant differences in the precise branching patterns and termination points identified, as well as a high dependency of the tractography findings on the settings used (Dauguet et al. 2007).

The minipig study described above also reported discrepancies between results from the two invasive tracer approaches (Dyrby et al. 2007). For example, injection into somatosensory cortex resulted in labeling of the ventral pallidum when using manganese but not when using BDA, reflecting manganese’s ability to track across synapses. This observation highlights the care that must be taken when considering a tracer technique a straightforward gold standard against which to compare tractography results. Tractography could, in principle, trace polysynaptic pathways, but in practice this will probably involve tracking through regions of low anisotropy, which may be problematic.

The direct validation studies further note that agreement among techniques varies from tract to tract. It is well known in the tractography literature that while some pathways are easy to track (e.g., corpus callosum), others are challenging (e.g., the lateral portion of the corticospinal tract, the auditory radiations), although improved techniques can improve performance (Behrens et al. 2007, Parker & Alexander 2005, Wedeen et al. 2008). Therefore, it is not possible to extrapolate good agreement on a particular tract of interest as general evidence for the validity of the technique.

Direct validation, as described above, is a major undertaking. Other, more indirect approaches can be performed more routinely and are complementary in providing a test for conclusions reached by tractography. For example, a number of studies have compared results based on tractography with those based on independent data from functional imaging. This is a particularly useful comparison when tractography has been used to parcellate a gray-matter structure into putative functional-anatomical subregions, such as the examples provided above for the thalamus (Behrens et al. 2003a) and the premotor cortex.
Using a meta-analysis of previously reported functional imaging activations within the thalamus, we found good agreement between the location of functional activations during motor tasks and the thalamic volume with high probability of connection to motor, premotor, and somatosensory cortex; similarly, activations during executive and memory tasks colocalized well with the region showing high probability of prefrontal connection (Johansen-Berg et al. 2005). This agreement increases our confidence that the parcellation defined by the tractography has functional relevance. In the cortex, we find good agreement between previously reported functional imaging activations and connectivity-defined parcellations of both premotor (Mayka et al. 2006, Tomassini et al. 2007) (Figure 3e) and cingulate (Beckmann et al. 2009) cortex.

Confidence in the maps provided by parcellation techniques is also increased by the uniformity in the boundaries that are defined across both scanning sessions within the same individual and across different subjects (Klein et al. 2007b, Tomassini et al. 2007). Nevertheless, small variations do persist in the positions of areal boundaries in different individuals once results are registered into the standard space that is typically used to represent data across subjects. These interindividual differences in structural boundaries seem to correlate in a straightforward and simple way with interindividual differences in the positions of functionally defined boundaries between brain areas. For example, when directly comparing functional and anatomical results in the same subjects, we find that the connectivity-defined border between supplementary motor area and pre-SMA colocalizes well with the functionally defined border between these two regions on the basis of fMRI during a motor and cognitive task (Johansen-Berg et al. 2004).

Other approaches to validation include comparison to cytoarchitectonic maps (Johansen-Berg et al. 2005, Klein et al. 2007b) and the use of structural MR images in which the contrast has been optimized to make aspects of brain structure, such as the positions of component nuclei of the thalamus, readily visible (Devlin et al. 2006).

CONCLUSIONS
Diffusion tractography provides a window on human brain anatomy that was previously unavailable. It therefore offers unique and exciting opportunities for novel research questions to be addressed both in the healthy brain and in the diseased brain. However, like any recently developed method, diffusion tractography requires careful validation and clear definition of its limitations and scope. We hope that researchers will take up the challenge of direct validation of this technique and that methodological developments in both image acquisition and analysis will continue to improve accuracy. Even the most sophisticated methods, however, will not place diffusion tractography in a position to replace conventional anatomical methods such as tracer studies in nonhuman animals. The exquisite detail and determination of tract polarity that is possible with active axonal tracer methods can never be achieved with diffusion imaging. Nevertheless, the ability of diffusion imaging to estimate anatomical connectivity across the whole brain, in a living human subject, places this technique in a unique position to test the relationship between brain structure and function and to complement evidence from conventional anatomical investigations.

DISCLOSURE STATEMENT
The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.
LITERATURE CITED


RELATED RESOURCES

FMRI B Diffusion Toolbox (FDT), FMRIB Software Library. 2009. DWI analysis and tractography. http://www.fmrib.ox.ac.uk/fsl
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