New Approaches for Exploring Anatomical and Functional Connectivity in the Human Brain

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Information processing in the primate brain is based on the complementary principles of modular and distributed information processing. The former emphasizes the specialization of functions within different brain areas. The latter emphasizes the massively parallel nature of brain networks and the fact that function also emerges from the flow of information between brain areas. The localization of function to specific brain areas (“functional segregation”) is the commonest approach to investigating function; however, an emerging, complementary approach (“functional integration”) describes function in terms of the information flow across networks of areas. Here, we highlight recent advances in neuroimaging methodology that have made it possible to investigate the anatomical architecture of networks in the living human brain with diffusion tensor imaging (DTI). We also highlight recent thinking on the ways in which functional imaging can be used to characterize information transmission across networks in the human brain (functional and effective connectivity).

Key Words: Diffusion, magnetic resonance imaging, functional connectivity, human

Advances in this field and give examples of how they can be used to determine aspects of the organization of the human brain.

A second, complementary approach is concerned with establishing the ways in which information is transmitted and integrated across brain networks. These are dynamic, context-dependent processes, in which variations in task demands lead to the preferential recruitment of some networks over others. Methods for analysis of these processes are based on the premise that functionally interacting regions will show correlated patterns of activity. Thus, simultaneously recording the activities of two groups of neurons in an animal preparation allows testing for conditions under which they become functionally coupled (Scannell et al 1995; Young et al 1994). The advantage of functional neuroimaging methods is that they can be used to detect activity not just in a limited set of areas but across the entire brain simultaneously. This makes it possible to examine the statistical relationships between the activities of not just two but of several areas across the brain. We will describe exciting new strategies for use of functional MRI (fMRI) data in the analysis of functional connectivity in the human brain. The review of diffusion tractography and functional mapping together highlights the possibility that future strategies for understanding interactions between regions of the human brain will benefit from integrating anatomically informed models of functional interactions.

Diffusion Tractography: Exploring the Connectional Architecture of the Human Brain

Recent advances in diffusion-weighted imaging and its derivative, diffusion tensor imaging (DTI), have brought to light the possibility of in vivo explorations of anatomical connectivity in the human brain. Magnetic resonance diffusion-weighted imaging sensitizes the nuclear MR signal to the random motion of water molecules along a single diffusion-encoding direction (Le Bihan 2003; Stejskal and Tanner 1965). By taking measurements along many such directions, it is possible to characterize the mean diffusion properties within a voxel. Diffusion tensor imaging then makes the assumption that this local diffusion might be explained by a three-dimensional Gaussian process and fits the diffusion tensor (Basser et al 1994) as its covariance matrix at each voxel. This tensor might be represented by a diffusion ellipsoid and, if the assumption of Gaussian diffusion holds true, the principal axis of this ellipsoid corresponds to the direction of greatest diffusion, or principal diffusion direction (PDD), and its

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prolateness or anisotropy corresponds to the degree to which diffusion is preferred along this direction over other directions.

In tissue with a high degree of directional organization, diffusion is more hindered in some directions than others (see Le Bihan 2003 for a recent review). For example, in white matter, the PDD corresponds well with the dominant orientation of fibres within the voxel (Beaulieu and Allen 1994). Therefore, by visualizing the field of PDDs measured by DTI we can estimate the local orientation of major white matter tracts at each voxel (Figure 1). In fact, the directional patterns of vector fields displayed graphically are so compelling that it is tempting to make specific inferences of the spatial trajectories of white matter tracts from them.

This process is based on two implicit assumptions. First, we assume that the underlying vector field along the pathways is “smoother” than the resolution of the diffusion-weighted image (i.e., that the fibre architecture in every voxel is well-represented by a single vector, and if we followed the true PDD, we would confidently follow the tract). Second, we assume the measured PDD field to be a faithful representation of the true PDD field (i.e., that noise has a negligible effect on the measured PDD).

In the case of a voxel in which fibre tracts fork into two pathways, a single vector might show the mean direction instead of describing the true direction of either fibre. Nonetheless, so-called “streamlining algorithms” (Basser et al 2000; Conturo et al 1999; Mori et al 1999) have been highly successful in reconstructing major fibre systems in the deep white matter (Figure 2; Catani et al 2002; Stieltjes et al 2001). There is a close correspondence between in vivo DTI-based reconstructions and information from postmortem studies; however, these approaches also have limitations. For example, they are only able to define paths when diffusion anisotropy is high. So, whereas large deep white matter paths are well-defined, pathways toward the neocortex, where there can be considerable fibre divergence, and hence low diffusion anisotropy, are not. Another limitation is that DTI-based reconstructions express results qualitatively and do not quantitatively measure the strength or confidence in the pathways described, which makes between-subject comparisons reliant on qualitative analyses.

Recent work has started to tackle the assumptions discussed above. The first assumption is a relatively difficult one to address. The voxel resolution in diffusion-weighted images is orders of magnitude greater than the diameter of an axon. As we seek to identify increasingly finer pathways, it will inevitably be the case that some areas are poorly described by Gaussian diffusion, because such areas might be poorly described in terms of a single dominant fibre direction. This topic is the focus of much current research. For example, Tuch et al (2003) propose techniques for recovering arbitrarily complex distributions on diffusion within each voxel. This allows the investigators to resolve areas of complex fibre structure, such as crossing fibres. Figure 3 is a typical example of the result of q-ball diffusion imaging in areas with complex fibre architecture, showing the potential for resolving more complex fibre structure within a voxel. Note the multiple lobes on the distributions, for example at the crossing of corona radiata and superior longitudinal fasciculus.

The second assumption is easier to tackle. By taking repeated measurements along the same diffusion-encoding directions and “bootstrapping” these data to create many DTI data sets, Jones (2003) was able to quantify the uncertainty in the measured PDD.
field. Uncertainty is typically low in deep white matter fibres, thus explaining the reproducibility of streamlining results in these areas; however, uncertainty is high in areas with geometrically complex structure (such as crossing fibres). Behrens et al (2003a) propose a method for estimating this uncertainty from a single data set and propagating this local uncertainty on PDD through to a global probability density function (PDF) on the recovered connecting streamlines. Thus, pathways seeded in a given location might encounter regions of high uncertainty as they approach their targets but be able to progress into target areas, with the PDF spreading spatially to account for the uncertainty earlier in the pathway. This is illustrated in Figure 4A, which depicts thalamo-cortical pathways (note the broadening of PDFs in the approach to the cortex; see figure legend for details).

An important advantage in computing a PDF on the location of the pathway is that it is possible to express a level of confidence in the resulting projection. Instead of discretizing the PDF on a voxel-by-voxel basis, Behrens et al (2003b) computed the probability of projection between seed points in the thalamus and each of seven anatomically defined cortical masks (Figure 4B). Seeds were classified according to the masked cortical region with which they had the greatest probability of connection (Figure 4C). Figure 4C (inset) shows a schematic diagram of the thalamus subdivided into histologically defined nuclei. Color

Figure 3. Q-ball diffusion imaging in the macaque monkey brain reveals fibre complexity within a voxel. Normalized orientational density functions are shown at each voxel. (A) A single coronal slice from macaque brain. (B) Detail of an area of crossing fibres in A (intersection of the superior longitudinal fasciculus, the corona radiata, and the corpus callosum). Image courtesy of D. Tuch.

Figure 4. (A–C) Probabilistic tractography between thalamus and cortex. (A) Probabilistic connectivity distribution from medio-dorsal thalamus to the prefrontal cortex and temporal lobe. (B) Anatomically defined cortical masks. (C) Axial section through thalamus showing result of connectivity analysis. Probabilistic tractography (see text) was run from each seed voxel in thalamus, and the seed voxel was labeled according to the cortical zone in B with the highest probability of connection. Inset: schematic of thalamus with (overlaid in color) predictions from the monkey literature of the dominant cortical connections within each thalamic nuclear cluster. (D–F) Probabilistic tractography between cerebral peduncle and cortex. Axial (D) and coronal (E) section through the cerebral peduncle, parcellated according to the highest probability of connection from cortical zones defined in F. A–C adapted from Behrens et al 2003a.
overlays represent predictions from the monkey literature of the
dominant cortical connection of each nuclear cluster. Figure 4C
(background image) shows the result of classifying seed voxels in
the thalamus to the cortical area with the highest probability of
connection. The authors examined the probability values of
connections between seed voxels and target areas and found
areas in the thalamus with a high probability of a connection to
more than one cortical mask and areas in which connection
probability is small with all cortical masks.

Establishing the validity of diffusion-based fibre tracking
methods is an important challenge, which can be accomplished
by evaluating both between-subject reproducibility and by com-
paring results with those from conventional tract-tracing methods
in the same brain. The former confers the advantage that
between-subject analyses can be quantitative, but it might be
complicated by the complexities associated with the spatial
warping of imaging data from several cases into a common
anatomical frame of reference (Xu et al 2003); deep white matter
tractography often relies on qualitative comparisons between
subjects. The latter is a particularly powerful approach to valida-
tion: nonhuman primate models might be used to demonstrate
that DTI and conventional tract-tracing methods reveal common
anatomical architecture when both methods are applied to the
same brains. It was recently reported that the uptake of manga-
enese through fibre tracts can be detected with MRI (Pautler et al
1998) and can therefore potentially be used as an anatomical
marker. The authors examined the probability values of
connections between seed voxels and target areas and found
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Functional and Effective Connectivity: Exploring
Models of Information Flow Through Networks

The functional mapping of different brain regions is the
primary approach to the analysis of functional imaging data.
Classic examples include the use of positron emission tomogra-
phy (PET) by Zeki et al (1991) to localize color and motion
centers of the human visual cortex (V4 and V5, respectively).
More recently, these analyses have been suggested by func-
tional integration analyses that describe how functionally
specialized regions interact with each other. This can be thought of
as the functional mapping of different brain pathways or net-
works. A recent example is the study by Buchel et al (1999), who
found that the success with which a subject learned an object–
location association task was correlated with the coupling be-
tween regions in the dorsal and ventral visual streams (Unger-
leider and Mishkin 1982).

Functional Connectivity

Early analyses of functional integration used principal com-
ponent analysis (PCA) to decompose neuroimaging data into a
set of modes that are mutually uncorrelated, both spatially and
temporally. The modes are also ordered according to the amount
of variance they explain. By comparing the temporal expression
of the first few modes with the variation in experimental task, a
distributed functional system associated with that task can be
identified (Friston et al 1993). A more sophisticated use of PCA
occurs in the context of generalized eigenimage analysis (Friston
1997), in which the principal component is found that is maxi-
mally expressed in one experimental condition/population and
minimally expressed in another (e.g., control vs. patient groups).

More recently, independent component analysis (ICA) has been
used to identify modes describing activity in a sparsely distrib-
uted network (McKeown et al 1998). Such PCA/ICA-based
methods are referred to as analyses of functional connectivity,
because they are data-driven transform methods that make no
assumptions about the underlying biology. They are therefore of
greatest practical benefit when it is not known which regions are
involved in a given task and/or what is the underlying structural
connectivity. In contrast, analyses of “effective connectivity”
(described below) are based on statistical models that make
anatomically motivated assumptions (e.g., knowledge of struc-
tural connectivity) and restrict their inferences to networks
comprising a number of preselected regions. These analyses are
hypothesis-driven rather than data-driven and are most applica-
able when one has knowledge of the relevant functional areas
(e.g., from analyses of functional specialization). Detailed discus-
sions of both approaches are found in Frackowiak et al (2003).

Structural Equation Modeling

Structural equation models (SEMs) were developed in the
field of econometrics and were first applied to neuroimaging
data by McIntosh and Gonzalez-Lima (1994). They comprise a set
of regions and a set of directed connections. Importantly, a
causal semantics is ascribed to these connections, whereby an
arrow from A to B means that A causes B. Causal relationships
are thus not inferred from the data but are assumed a priori (see
Figure 5).

An SEM with particular connection strengths implies a partic-
ular set of instantaneous correlations between regions. One can
therefore set the connection strengths so as to minimize the
discrepancy between the observed and implied correlations and
thereby fit a model to data.

If, for example, one partitions a given fMRI data set into those
scans obtained under two different levels of an experimental
factor, then one can attribute differences in connectivity to that
factor and so conclude that a pathway has been activated.
Structural equation models have, to date, been the most widely
used model for connectivity analyses in neuroimaging (see
Goncalves and Hull 2003), and we envisage that this will remain
the case for experiments in which PET data are used.

There are, however, two major drawbacks to SEM. First,
because SEM only makes use of information in correlation
matrices, it is only possible to specify networks with a limited

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number of connections. Such sparse structures that neglect, for example, reciprocal connections between regions are often biologically implausible and might result in poor model fits. Second, SEMs do not make use of temporal information—if the time indexes of the data were randomly permuted, SEM would give the same results.

**Multivariate Autoregressive Modeling**

To overcome these difficulties, Harrison et al (2003) have proposed the use of multivariate autoregressive (MAR) models for the analysis of fMRI data. An autoregressive approach is used to characterize structure in a time series, whereby the current value of a time series is modeled as a weighted linear sum of previous values. Multivariate autoregressive models extend this approach to multiple time series, so that the vector of current values of all regions is modeled as a linear sum of previous vector values. The optimal number of preceding time points can be found with Bayesian model order selection (see Figure 6).

In a MAR model, the dependencies between time points and between regions are characterized by a matrix of weighting values. Estimation of these weights is a noniterative linear fitting process. Thus, estimation is fast, which opens up the possibility of readily comparing connectivity models comprising different regions and connectivity patterns.

The parameters of a MAR model can be used to compute a number of further quantities, each of which can be used to describe network connectivity. These include coherences (correlation at particular frequencies), partial coherences (the coherence between two time series after the effects of others have been taken into account), phase relationships (the lag between two signals at a given frequency), and Granger causality (the dependence of region A on region B, as assessed by comparing two MAR models, one with the A-to-B connection and one without).

By partitioning an fMRI data set into different levels of a factor, one can then infer that pathways have been activated or that, for example, Granger causality between regions has changed. Multivariate autoregressive models are only beginning to be applied in fMRI but have a history of application in electroencephalography (EEG)/magnetoencephalography (Bressler and Scott Kelso 2001).

**Dynamic Causal Modeling**

Whereas SEM and MAR models were developed in other areas of science, dynamic causal modeling (DCM) (Friston et al 2003) has been specifically designed for the analysis of functional imaging data. Dynamic causal modeling posits a causal model, whereby neuronal activity in a given region causes changes in neuronal activity in other regions, via interregional connections, and in its own activity, via self-connections (see Figure 7). The neuronal activity in each region then gives rise to changes in blood volume, flow, and deoxyhemoglobin content. These then determine the blood oxygen level–dependent signal that is measured with fMRI. In DCM, these hemodynamic relationships are quantified by the Balloon model (Friston et al 2003).

Thus, DCM models neuronal connectivity, whereas SEM and MAR model correlations at the level of observed fMRI time series. Dynamic causal models are able to work at the neuronal level because they use a “forward model” (with hemodynamic parameters), relating neuronal activity to fMRI activity, and this model is
inverted during the model-fitting process. Another important distinction is that DCM explicitly models the effect of experimental manipulation on network dynamics. Mathematically, neuronal activity is described by a bilinear differential equation, whereby transient responses are initiated via driving external inputs, and the time constants of these transients can be altered via modulatory inputs. The strength of these driving and modulatory input connections (or "neurodynamic parameters") can be estimated from data.

A DCM is fitted to data by tuning the neurodynamic and hemodynamic parameters so as to minimize the discrepancy between predicted and observed fMRI time series. This takes place via an iterative nonlinear fitting process. A current limitation of DCM is that, because this model fitting is computationally demanding, one must restrict analyses to a small number of regions.

An example of an analysis with DCM is a study of whether category specificity effects in infero-temporal cortex are mediated by top-down or bottom-up activity (Mechelli et al 2003). We anticipate that the DCM approach rapidly will become widely used because it both 1) explicitly models how experimental manipulations cause network activity; and 2) models this activity at a neuronal rather than hemodynamic level, a level that is most appropriate for understanding information flow.

A second current limitation of DCM is that neurodynamics in each region are characterized by a single state variable ("neuronal activity"). This prohibits inferences that can be meaningfully linked to specific neurotransmitter systems, because these would require multiple state variables in each region that describe activity in excitatory and inhibitory subpopulations. The parameters of such models could only be identified with DCMs that use high temporal resolution data, such as from EEG. The development of such models therefore requires integration of information from fMRI (to determine where activity occurs) and from EEG (to determine when it occurs) and is an exciting area for future research that would significantly strengthen the bridge between data from imaging neuroscience and our understanding of the neurobiology underlying cognitive processing.

A Multidisciplinary Approach to Understanding Connectivity

This review has provided an overview of recently developed methods that permit investigations of anatomical and functional connectivity in the human brain. Although these methods have yet to reach the peak of their sophistication, it is clear that they have already made significant contributions to our understanding of how the human brain operates as a collection of networks. The same methods also promise to transform the ways in which we think about the underlying causes of neuropsychiatric conditions. For example, DTI has been useful in the identification of connectional abnormalities in fronto-parietal and fronto-temporal circuitry in schizophrenia (Burns et al 2003; see Lim and Helpern 2002 for a review). Investigations of functional connectivity have been useful in studies of neurotransmitter systems closely linked to schizophrenia. As a recent example, Honey et al (2003) demonstrated that dopaminergic drugs alter the functional connectivity between areas of the prefrontal cortex and interconnected regions of the striatum and thalamus.

In future work, the combined use of DTI and functional connectivity analyses will also serve to overcome important limitations. Methods for examining effective connectivity (e.g., DCM, as described above) often require the a priori specification of anatomical connectivity models in the system of interest, but...
these are inevitably inaccurate because they are derived from nonhuman primate studies, and the connectivity between areas in the human brain is almost always unknown. The methods described here offer the prospect of using DTI to specify the anatomical model to inform functional connectivity analyses, not only in the same species but also in the same subjects.

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