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How our brains are wired: Are the applications of diffusion imaging useful given the current limitations?

Review

Diffusion imaging (DI) enables researchers to study white matter (WM) pathways in the human brain in-vivo by labelling water molecules and measuring their diffusion into different directions. Connectivity patterns are inferred assuming that water diffuses rather along than across fibre bundles. This paper introduces the concept of DI, addresses suitable applications and evaluates gains versus limitations. Common applications are (1) generating WM atlases, (2) mapping connectional models of functionally subdivided brain regions, (3) linking disorders to connectivity abnormalities, (4) verifying WM pathways from animal studies, (5) linking personality traits to particular connectivity patterns, (6) measuring structural changes resulting from experience or ageing and (7) presurgical planning. Despite limitations like the moderate spatial resolution, or – more fundamentally – the lack of a gold standard and the kissing/crossing problem, DI can be regarded as a useful tool if researchers choose methods carefully and consider the known limitations.
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INTRODUCTION

Diffusion imaging (DI) has become popular over the last decades as an effective tool to measure water diffusion in the human brain in-vivo in order to infer white matter (WM) connectivity patterns. Due to its unique possibilities, several new

applications - like the human connectome project – have arisen. Initiated in 2009 by the United States' National Institutes of Health (NIH), the human connectome project aims at mapping the complete structural and functional neural connectivity of the human brain within five years. One of the program's goals is to optimize DI techniques and to improve and validate methods of analysis. Special software that can deal with such a great amount of data is required as well, which poses a great challenge to the research teams from nine different institutions that are involved in the project. If a way is found to map all the connections and to structure them conveniently and in an easily accessible way, new possibilities for studying relations between neural connectivity and neurological and psychiatric disorders arise (NIH Human Connectome Project, 2010).

Many more new possibilities have emerged out of DI. The current article gives an outline of how DI works and how the DI data can be visualized. Further, tractography which is used to reconstruct fibre pathways is introduced. Afterwards, methodological and technical limitations of in DI are specified. In the last section, a number of applications is presented and it is discussed whether they are useful given the earlier introduced limitations of DI in general and the different models and algorithms in particular.

DATA REPRESENTATION IN DIFFUSION IMAGING

The Underlying Principle

At room or body temperature, water molecules move due to thermal energy, a phenomenon that is called Brownian motion (Einstein, 1956). If they move equally into all directions, this is called isotropic diffusion. In the brain, however, water diffusion is restricted by tissue barriers and anisotropic (Beaulieu, 2002). It is assumed that water molecules diffuse along axons rather than perpendicular to them (Johansen-Berg & Rushworth, 2009). Consequently, measuring diffusion with Magnetic Resonance Imaging (MRI) allows to infer the location of WM bundles.

The MRI scanner can be used to generate anatomical (MRI), functional (fMRI), and diffusion images of the brain. These different modalities are possible because the scanner can be tuned by using different scanning sequences. Standard MRI uses the fact that hydrogen atoms behave differently in different tissue types to reconstruct anatomical images. Functional MRI uses sequences that are sensitive to blood water. Depending on whether it contains more or less oxygen, it has different magnetic properties that can be distinguished with MRI and we assume that more oxygenated blood flows to regions of higher activation. To pick up diffusion, two detection radiofrequency (RF) pulses are introduced shortly after each other which allows to see whether and where water has moved (Gazzaniga, Ivry & Mangun, 2008). Several different models can be applied to analyse and visualize the DI data subsequently. Diffusion Tensor Imaging (DTI) is the most common model. In the following section different models will be introduced, each of which tries to overcome different constraints and limitations.

The Diffusion Tensor Model

In DI, one acquires many images that are diffusion weighted into different directions. So the MRI scanner uses RF pulse sequences tuned consecutively to diffusion into different directions, among them the three main directions x, y and z, but also directions in between. For DTI, diffusion information in optimally 60 or more directions is required and information from these differently weighted images is combined to calculate the so-called diffusion tensor, which is then used to reconstruct the DTI image. The diffusion tensor indicates how strong diffusion happens in each direction. The more spheric the tensor is the more water diffuses equally into all three directions while a more compressed tensor indicates directional dependency. Three quantitative measures can be reported for each voxel: The principal diffusion direction - the diffusion direction with the highest eigenvalue (Johansen-Berg & Rushworth, 2009), fractional anisotropy (FA) and mean diffusivity (MD). They indicate respectively local fibre orientation, the degree of directionality (FA) and the strength of the water diffusion (MD). FA describes thus the extent of anisotropy and ranges from zero (isotropic, e.g. in cerebrospinal fluid (CSF)) to one (completely anisotropic). Figure 1 nicely illustrates the relation between FA and the diffusion tensor. If the tensor is more spherical FA is lower because water diffuses with equal probabability into all directions (Johansen-Berg & Rushworth, 2009). FA is widely used its independence of local fibre orientation makes it a suitable measure to compare tract integrity across subjects (Smith et al., 2006).

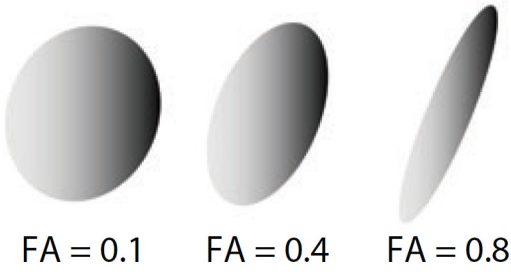

Figure 1. Relationship between diffusion tensor and FA values.
With permission from Johansen-Berg & Rushworth, 2009.

MD gives information about the strength of the diffusion in each voxel, regardless its directional dependency. For example, MD values are high in CSF but low in gray matter while FA values are low for both, since these regions typically show no strong directional dependent diffusion. With DTI data it is not possible to map several diffusion directions within one voxel because it only offers the principal diffusion direction. Notwithstanding, DTI is widely used and especially useful in regions with single fibre orientation where no crossings distort the results.

Multiple Direction Approaches

Multiple direction approaches are advantageous because they are able to map intra-voxel diffusion heterogeneity. Compared to DTI, not only the principal diffusion direction but two or more directions of water diffusion are reported for each voxel. Further, the full diffusion tensor is used instead of only the principal direction in DTI. A drawback is that a much larger number of images from different angles is required, which increases scanning time (Hagmann et al., 2010). Examples are High Angular Resolution Diffusion Imaging (HARDI) or Diffusion Spectrum Imaging (DSI). They differ only slightly and for our purpose it is enough to say that they use different filters when analyzing the DI data (Wedeen et al., 2008).

Evidence for the ability of DSI to reconstruct tracts adequately comes from studies that used fixed monkey brains and compared the results of DSI and DTI (Wedeen et al., 2008) or autoradiographic tract-tracing (Schmahmann et al., 2007). Autoradiographic tract tracing allows to identify fibre tracts based on stained histological data. DSI resolution was lower than in the autoradiographic tract tracing, but fibres that crossed with an angle larger than 15° could be resolved (Schmahmann et al., 2007). Even in gray matter and subcortical nuclei, crossing fibres could be disentangled using DSI (Wedeen et al., 2008). Figure 2 illustrates the superiority of DSI (C) over DTI (D) in its ability to detect fibre pathways. Despite the fact that Schmahman et al. (2007) evaluated DSI using a fixed monkey brain instead of making in-vivo measurement, these results are promising for the technique in general. Notwithstanding, the main limitation of DSI is that it is very time consuming and expensive because of the large number of images that needs to be sampled (Wedeen et al., 2008; Hagmann et al., 2010). Also the longer scanning times increase the risk for noise introduced by patient movement.

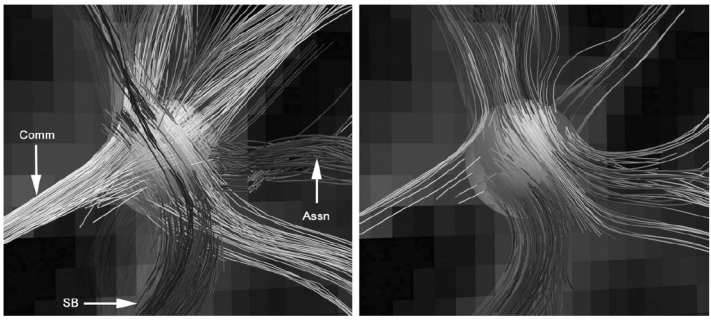

Figure 2. Comparison of DSI (C) and DTI (D) in mapping fibre pathways of a fixed monkey brain. With permission from Wedeen et al., 2008.

In sum, DTI is simple and straightforward in visualizing DW data and especially good for regions with single fibre orientations. Whereas multiple direction models are useful if a-priori knowledge suggests that the regions of interest (ROIs) contain a lot of touching (kissing) and crossing fibres that are hard to distinguish. Particularly, DSI has proven to be able to identify the same major pathways that were found with tracing methods (Schmahmann et al., 2007).

FIBRE TRACKING

After a model has been fit, specific pathways can be identified with the help of fibre tracking algorithms during a process called tractography. The tractography reconstructs WM bundles either in a deterministic or in a probabilistic way.

Deterministic versus Probabilistic

In Deterministic tracking a streamline is created along the principal diffusion direction starting from a seed ROI to identify the tract of interest (Hagmann et al., 2010). Extra information can be added to stop tracking, e.g. when FA, MD or the angle fall below a certain threshold. Figure 3 illustrates how the streamline runs along the tensors with the highest FA values.

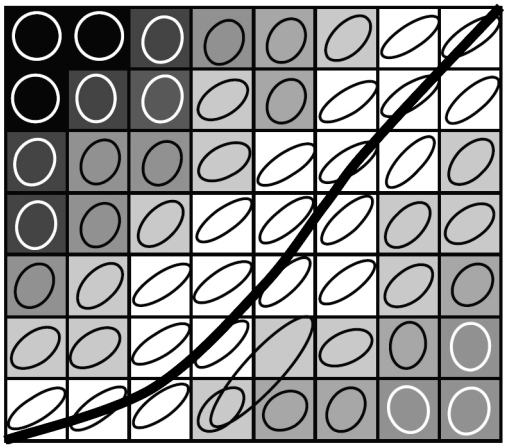

Figure 3. Simple Streamline Tractography. With permission from Johansen-Berg & Rushworth (2009).

Deterministic tracking thus gives for any particular seed the point of destination that is most likely. Especially in combination with a-priori knowledge about anatomic and functional ROIs, deterministic tractography provides good estimates of the neural connectivity.

On the other hand, probabilistic tracking focuses on the likelihood of anatomical connections between two points and offers a good way to overcome uncertainty (Wedeen et al., 2008). For any particular seed a 3D map of connectivity probability is given instead of only one terminal point (Roebroeck et al., 2008). This is done by sampling thousands of streamlines originating from the seed rather than one line along the principal direction. The criteria for creating these streamlines are similar to deterministic tractography in the sense that FA, MD and angular thresholds can be implemented, too. But while deterministic tractography allows only one connection from one voxel to the next, probabilistic tractography follows several possible streamlines at the same time if uncertainty arises about which tract

to follow. The huge number of gathered streamlines is then analyzed and the density of lines reflects the probability of neural connections (Johansen-Berg & Rushworth, 2009). In addition, waypoints and exclusion points can help to limit the amount of identified tracts. Like the seed region, they need to be drawn manually. Waypoints are defined as points where every tract needs to run through, while exclusion masks define regions not to be included. A disadvantage of probabilistic tracking is that small levels of diffusion in different directions than along the axons will influence the analysis. This can require more smoothing and distort the results so that some fibre pathways are reconstructed inaccurately (Descoteaux, Deriche, Knösche & Anwander, 2009).

Conclusively, although probabilistic tracking requires longer calculation times, one advantage over deterministic tractography is that it allows to continue tracking if uncertainty arises, e.g. if tracts are really curvy (Johansen-Berg & Rushworth, 2009). Still, if hypotheses have been formed in advance deterministic tracking is superior to probabilistic tracking because it is more straightforward and does not show WM pathways in unexpected regions. Therefore, deterministic tracking should be preferred if knowledge and hypotheses about ROIs are available because then it provides more realistically reconstructed pathways.

Comparing Groups

DI can also be used to compare WM structures between groups. There are two major approaches to do so. First, ROI approaches and tract-specific measurements only investigate group differences within the ROI, and therefore they require prior knowledge about the regions that are thought to differ (Catani, 2006). Secondly, whole brain Voxel Based Analysis (VBA) approaches allow group comparisons based on the entire brain volume. They have the advantage that they are fully automated and do not rely on a-priori assumptions. Further, it was found that VBA was able to identify more correlations with age than a ROI approach, which is probably caused by the averaging in ROI approaches (Snook, Plewes & Beaulieu, 2007).

If one group is found to have higher FA values this can have two different implications. Either it indicates a stronger connection strength, which holds especially in regions of unambiguous fibre pathways, or it indicates higher directional coherence in regions of fibre crossings (Rouw & Scholte, 2007). Tract statistics are one way to compare groups and it can be done in a deterministic way, for instance based on streamline tractography (Capalbo, 2008), or in a probabilistic way, as it is the case in tract based spatial statistics (TBSS). TBSS offers a whole brain voxel based approach and has many benefits – like reducing the amount of false positive connections caused by misalignment in other VBAs – but it also introduces problems, like rising the amount of false negatives because it only looks at the WM tracts that have been included in the analysis (Hsu et al., 2008). Some examples of comparing groups are discussed in the applications section of this paper.

LIMITATIONS OF DIFFUSION IMAGING TECHNIQUES

Technical and Methodological Constraints

Different models and fibre tracking algorithms have different technical and methodological constraints. One typical limitation is limited spatial resolution. While an axon bundle is estimated to have a diameter of about 1mm, the spatial resolution of DI lies approximately between 3 and 15mm^3, depending on the applied model (Descoteaux et al., 2008). Moreover, it is not yet clear where FA values actually are derived from. The reliability of FA values might be decreased by factors like large axon diameter. More importantly, when a signal is measured it is never sure what it actually reflects because there is no one-to-one relationship between FA and WM strength (Johansen-Berg & Rushworth, 2009). Fortunately, new tractography and registration methods are developing quite fast and at least problems like low resolution are being improved already. A study by Roebroeck and colleagues (2008) showed that heightened resolution can be reached by higher field strengths and this elevates the accuracy of DTI-based tractography since the principle diffusion directions would be described more accurately. However, higher field strengths introduces other problems like higher safety requirements or increased noise caused for instance by more susceptibility to magnetic materials, that might be contained among others in make-up. Furthermore, the human connectome project also aims at improving scanner technology and resolution (NIH Human Connectome Project, 2010).

Fundamental Limitations

Nevertheless, other limitations of DI techniques are more fundamental in the sense that they are inherent to the method and cannot be improved as technology progresses. One is the lack of a gold standard where the results of different approaches can be compared to. Although data can be simulated using phantoms and compare the DI data thereto, this does not take into account imaging artefacts, limitations due to voxel size, and scanner noise (Campbell et al., 2005; Tournier et al., 2008). One study that presents a promising beginning for the validation process compared WM tracts of the rhesus monkey brain reconstructed with DSI to data derived by autoradiographic tract tracing. The result was promising and the main fibre tracts identified with DSI matched the findings from the histological investigation (Schmahmann et al., 2007).

Another fundamental limitation of DI is the kiss-crossing problem. Initially, it was mainly about the fact that DTI is not able to detect fibre crossings within single voxels. This challenged the interpretability of fibre tracts reconstructed with DTI, especially because it has been shown now that about one third of the voxels contains two different fibre orientations (Behrens, Johansen-Berg, Jbabdi, Rushworth & Woolrich, 2007). Today, HARDI techniques or multiple-direction models allow to address the crossing problem and identify orientation distribution functions (ODF), which are functions that describe several principal diffusion directions within a single voxel (Fritzsche, Laun, Meinzer & Stieltjes, 2010, Schmahmann et al., 2007). Still, it is only feasible to show that there is more than one orientation of

water diffusion within a voxel, but not to distinguish between crossing fibres and fibres that are touching ("kissing") each other (Tuch, 2004). Therefore, the fibre crossing problem was reformulated the kiss-crossing problem and this is what can still be called a fundamental limitation of DI.

Moreover, DI studies often rely on anatomical a-priori information to define seed ROIs and are only as good as the techniques used to gain the a-priori knowledge (Catani, 2006). Assuming a strong relationship between functional activity and connectivity as well, one can use functional rather than anatomical knowledge to determine the ROIs to investigate connections originating in the higher visual areas V5 and MT (Lanyon et al., 2009). They performed fMRI analysis to identify the functional ROIs before they performed deterministic tractography with the DI data. By taking functional information into account error induction due to interindividual differences can be reduced. Yet, it is important to bear in mind that there is no one to one relationship between functional activity and connectivity.

Finally, there is uncertainty about whether reconstructed fibre tracts are afferent or efferent, which can only be known by the use of tracers on the axonal level. Moreover, it can never be known with certainty whether there are connections present or not at all. A definite conclusion cannot be drawn by means of present DI studies since they measure connectivity only indirectly via water diffusion patterns (Johansen-Berg & Rushworth, 2009).

APPLICATIONS

DI techniques offer the possibility for new research since it has been impossible to investigate WM pathways in vivo before. This section introduces current applications of DI, starting with the human connectome, which is an ambitious attempt to generate an exhaustive WM atlas.

The Human Connectome

In 2009, the United States' National Institute of Health (NIH) initiated the human connectome project with the goal to map the complete structural and functional neural connectivity of the human brain by multimodal scanning of 1200 brains of healthy adults. Such an atlas of WM tracts connecting brain regions could be useful in several fields of cognitive neuroscience. It would enable researchers to study relations between neural connectivity and neurological disorders in a novel way. The knowledge about structural and functional connections within and across individuals could be used to identify abnormalities linked to particular disorders, like Alzheimer's or Schizophrenia, and this permits addressing those disorders from another direction. Nevertheless, identifying the connectivity patterns of the whole brain produces a great amount of data. The analysis of this data has to be done in a careful manner so that a large-scale model of the neural connectivity can be achieved. The process of structuring the data gained with DI in a manageable and convenient way poses a great challenge.

Large-Scale Connectional Models

Large-scale connectional models are models of the fibre connectivity patterns in the human brain based on empirical findings and try to infer connectivity patterns of functionally subdivided brain regions (Catani, 2006). These models use ROI based approaches to identify networks that can be linked to functions. For instance, Capalbo (2008) mapped the structure of the human visual system by applying DTI to functionally relevant regions that had been identified a-priori. Evidence for the reliability of the reported large-scale model comes from agreements between subjects and congruence with a monkey connectivity matrix derived from tracing studies.

Hodolocial Syndromes

When cognitive and behavioural dysfunctions can be related to hyper- or hypo-connectivity between certain brain regions rather than due to damage to the brain regions themselves, they can be called hodological disorders (termed by Catani, 2006). DI offers a tool to investigate underlying connection mechanisms and to detect pathological WM pathways (Catani, 2006). For instance, an increased risk for Alzheimer's has been found to be correlated with significantly different FA values in preclinical stages e.g. in the uncinate fasciculus (Taoka et al., 2006). Furthermore, attention deficit hyperactivity disorder (ADHD) (Silk, Vance, Rinehart, Bradshaw & Cunnington, 2009) and Huntington's disease (HD) (Reading et al., 2005; Weaver et al., 2009) have been associated with abnormal WM development. A whole brain voxel based approach was used to compare presymptomatic HD patients with a matched group of healthy controls. Presymptomatic HD patients could be identified by testing for a HD gene mutation. The results showed significantly decreased FA values in clusters of the frontal lobe for the pre-symptomatic subjects ($p<0.005$) (Reading et al., 2005).

Verify WM Pathways Identified in Animal Studies

Human DI data can be compared to data of nonhuman primates that was gathered with invasive tract tracing like anterograde or retrograde tracing. Antero- and retrograde tract tracing offers information about whether connections are afferent or efferent. So we can transfer this knowledge about afferents or efferent tracts from animal studies to human DI data and circumvent this limitation of DI.

Further, Catani (2006) states that "verifying the existence of pathways described in animals and identifying possible tracts that are unique to humans belongs to the most essential purposes of DI" (p.3). A study that aimed at verifying findings of prior animal research was done by Leh, Ptito, Chakravarty & Strafella (2007). The researchers investigated cortico-striatal projections with probabilistic tractography and tried to find evidence for one of two hypotheses: While the information funneling hypothesis states that cortico-striatal projections are rather convergent, the parallel processing hypothesis claims that functionally distinct areas of the cortex have segregated projections to the striatum. They found that functionally related anatomical subdivisions of the striatum in humans projected to different cortical areas, which supports the information funneling hypothesis. Until

then, this had only been investigated by animal studies and evidence was yielded by some fMRI and rTMS studies. Yet, measures of the structural connectivity can only be gathered with the help of DI.

A link between WM structures and personality

Recently, correlations between WM structures and personality have been investigated. For instance, Cohen, Schoene- Bake, Elger & Weber (2009) explored whether personality traits are associated with WM integrity. Novelty seeking and reward dependence, which are generally linked to striatal activity have been measured with self-report questionnaires. Then, the DI images, acquired with HARDI, were aligned to a standard space to enable comparison. Indeed, connectivity maps of the striatum and scores on the questionnaires were linked. Statistical analysis revealed that high novelty seekers had stronger connecions from striatum to hippocampus and amygdala ($p< 0.05$) compared to high self-reported reward dependence, which in turn showed stronger connections between striatum and a distinct cortical network including areas in the orbitofrontal and prefrontal cortex (Cohen et al., 2009).

Until now it had not been possible to link personality to connectivity patterns. Especially in the field of behavioural and neuroeconomics where neural substrates of human decision making are investigated this offers new possibilities, like linking human decision making to structural connectivity patterns. In addition to personality traits, intelligence has been addressed by DI studies as well. Chiang and colleagues (2009) sampled FA values of twins and compared them to verbal, performance and full-scale intelligence quotients derived from the Multidimensional Aptitude Battery (MAB). The researchers aligned all diffusion images to a common space – similar to the TBSS procedure described earlier – in order to make the subjects comparable. They found a significant ($p < .05$) correlation of up to .4 between FA values and IQ scores – except for verbal IQ. Furthermore, Chiang and colleagues (2009) investigated heritability and found that the same genetic influences may affect both WM integrity and intelligence, which suggests a common underlying mechanism. This study is a good example of how DI can be used to offer new approaches to the study of intelligence and heritability.

Experience Dependent WM Changes

DI has also been used to detect experience dependent changes in WM. For instance, one study investigated the effect of juggling on WM using DTI and TBSS (Scholz, Klein, Behrens & Johansen-Berg, 2009). TBSS enabled the researchers to perform a whole brain analysis and to compare WM integrity of the group before and after the juggling period. The results showed that six weeks of juggling training already led to significant WM changes, which did not occur in the control group.

The structural changes did not correlate with performance levels after training. Rather, they seem to reflect the time spent juggling. The study nicely illustrates that the combination of DTI and TBSS is not only useful in investigating changes between groups, but also in detecting changes within groups over time. Obviously, the results indicate that behaviour has influence on the neural pathways

and the connectivity patterns in the brain. Since six weeks of training already induced WM changes one could suggest to access the effectiveness of therapy or medication with DI as well, e.g. in ADHD which has been linked to WM abnormalities (Silk et al., 2009). So, DI offers a good way to test to which extent behaviour and experience can change structural and functional connections in the brain.

WM Changes with Age

DI studies have also addressed age related changes in WM. For instance, a whole brain approach revealed that several regions – e.g. the anterior corpus callosum – show a negative correlation between FA and age (Hsu et al., 2008). The choice for reporting FA values is based on the fact that FA is widely accepted to be good for intersubject comparisons. Their findings are consistent with other studies and suggest that fibre strength decreases with age (Thomas et al., 2008, Charlton, Schiavone, Barric, Morris & Markus, 2010). A DTI study investigated whether the age-related changes can be linked to cognition (Charlton et al., 2010). Indeed, they found a correlation between decreased working memory capacities and reduced WM integrity as indicated by MD. The fact that MD and not FA was found to correlate with working memory changes may be due to the fact that MD is a more global and homogenous measure of WM strength rather than directionality (Charlton et al., 2010).

Similarly, Thomas et al. (2008) investigated the relation between WM changes and age-dependent decreases in the ability to discriminate faces. The subjects had to judge whether two faces were the same or different. Accuracy – not RT – was then compared across the subjects ranging from twenty to ninety years of age and revealed that the ability to discriminate faces declined with age. Deterministic tracking of the inferior fronto-occipito fasciculus (IFOF) and the inferior longitudinal fasciculus (ILF), two regions associated with face recognition, revealed age-related declines of FA as well as a hemispheric asymmetry implying that the right IFOF is more affected by age than the left IFOF and the ILF. It has been suggested that the right occipito-temproal regions are crucial for face discrimination since damage to this region impairs this ability (Meadows, 1974). This finding supports the link between the right IFOF and the ability to discriminate faces. A positive correlation was also found between FA in the right IFOF and accuracy, meaning that lower FA values were associated with higher error rates (Thomas et al., 2008). All in all, these studies are consistent in showing that increased age goes together with decreased FA/MD and there is evidence for associations between cognitive functioning and WM integrity.

Presurgical Planning

DI can also be used to identify specific pathways prior to brain surgeries. One of the pathways that have to be identified regularly is the optic radiation (OR), a WM bundle in the human visual system connecting the lateral geniculate nucleus (LGN) and the primary visual cortex (V1). The OR is of particular interest because it runs through the temporal lobe which is often dissected in epilepsy patients and its damage can lead to visual-field deficits. Further, the OR shows significant variability between subjects (e.g. Nilsson et al., 2007). This increases the need to identify it

prior to surgery. Diffusion imaging is the first in-vivo method to identify the OR and measure distances to other anatomical landmarks. Linear regression analysis has shown that visual field deficits can be predicted by the distance between two landmarks of the OR, Meyer's loop and the temporal pole. This is good evidence for the effectiveness of presurgical tractography of the OR to decrease risk of visual field deficits (Yogarajah et al., 2009).

DISCUSSION

The aim of this article was to introduce DI and to discuss advantages and disadvantages of the available models and algorithms. Subsequently, several applications have been addressed to show the importance and uniqueness of DI. The final question is whether the applications are useful and how DI can be used to improve further research. To start with, there are several reasons to assume that DI data is reliable. Support comes from the congruence between the DSI/DTI derived data and tracing studies in animals. Furthermore, the fact that DSI and DTI were able to detect major fibre pathways that have been found in animals before supports the reliability of DI even more (Schmahmann et al., 2007). Still, the question that arises is whether and when DI can be considered useful despite methodological and technical limitations.

In terms of the human connectome project, DI offers stunning possibilities. If the aim to improve methods and technology and to validate DI data is fulfilled, it may contribute to the further usefulness and reputation of DI. However, the amount of data to be collected is huge and good organization and visualisation techniques are required in order to benefit from it. Also in terms of large-scale connectional models promising possibilities arise. While Capalbo (2008) generated a functional model of the visual system, one of the best understood systems in the brain, future research might be able to address other sensory systems like audition or touch as soon as they are better understood. This might shed light on how senses are integrated and can have implications for the understanding of how we perceive the world around us.

Furthermore, DI provides a way to study causes of hodological syndromes. It is encouraging that different studies were able to find significantly different FA values in some frontal regions in presymptomatic HD patients compared to controls (Reading et al., 2005, Weaver et al., 2009). Although it is not yet clear what those FA changes actually reflect, the value of this application lies in the fact that DI might eventually contribute by identifying illnesses before other diagnostic tools are able to do so. We do not yet know whether FA changes precede other changes associated with specific diseases, like cell death. Yet, it is worth to investigate this, since this would make DI a useful and new diagnostic tool in the future.

The verification of neural pathways that have been found with tracing in animal studies can test hypotheses about brain circuits that have only been based on animal research before. Leh et al. (2007) found evidence for a hypothesis about cortico-striatal projections by using probabilistic tractography. The choice for probabilistic tracking can be explained by the fact that the researchers did not

want to constrain the tractography by using deterministic tracking and a-priori information that could have influenced in favour of one of the two hypotheses. This is another example of how the pro's and con's of the different methods and algorithms have to be balanced in order to guarantee a useful DI application. Personality has also been investigated with the help of DI and deterministic tracking and the technique has proven to be useful as it allowed researchers to correlate FA values and personality traits (e.g. Cohen et al., 2009).

Finally, DI experiments were able to show that experience and ageing influence WM structure as well. For instance, in the juggling study behaviour shaped WM (Scholz et al., 2009). The fact that DI is able to investigate behaviour induced WM changes leads to possible future applications like to investigate whether WM abnormalities decrease or even vanish in ADHD children as a result of reduction of the symptoms after therapy or medication. Finally, apart from research related applications, DI has also proven to be a useful tool in the clinical routine. Tractography in presurgical planning can be used to identify structures that are at risk of being damaged.

For the future, DI might be of use in lesion studies, which try to link cognitive or behavioural deficits to specific brain damages and therewith aim to find out whether an area is necessary for a particular function. A frequently encountered problem in lesion studies is that an investigation with functional magnetic resonance imaging (fMRI) only measures whether a region is intact or not. If the region is active and there is a cognitive or behavioural deficit, the region is not assumed to be necessary for this particular function, but it might be that this region is disconnected from other regions it usually interacts with. So it would still be linked to the observed deficit. Therefore, it would be valuable to combine lesion studies with DI techniques in the future, to get a more complete picture.

To sum up, DI has proven to be a useful tool in the cognitive neurosciences. Its benefits lie not only in its ability to reconstruct WM pathways in vivo, but also in the new methodologies that allow us to compare WM structure between groups or within groups over time. Furthermore, research also offers more and more evidence for DI's reliability, like the congruence between tracing and DSI when studying monkey brains. Even if there remain several limitations like the lack of a gold standard or the kiss-crossing problem the advantages outperform these limitations in most cases. The vagueness with regard to the exact meaning of FA also needs to be addressed in more detail in the future since FA is widely used for statistical comparisons. Yet, for current purposes DI provides useful new insights and methods are improving so that limitations like not being able to detect intravoxel diffusion heterogeneity soon will not be a problem anymore. Therefore it can be concluded that DI offers useful and more and more reliable results if the right methods and algorithm are chosen.

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