Biological Mechanisms of Dynamic White Matter Alterations

Do Tromp[1]

[1]Neuroscience Training Program, University of Wisconsin, Madison, WI 53703

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Abstract

Although the adult brain is perceived as largely fixed, this concept is receiving increased scrutiny. One way in which the brain can dynamically change in response to repeated stimuli is by altering the speed of conductivity. Oligodendrocytes are a major cell type allowing for the increased or reduced insulation of neuronal axons, through alterations in myelination. One novel way to measure these changes is through the non-invasive method of diffusion magnetic resonance imaging. This article will provide an overview of the current state of research, and provide clues as to how oligodendrocytes are mediating dynamic white matter alterations.

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BIOLOGICAL SCIENCES

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CORRESPONDENCE:
do.tromp@gmail.com

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INTRODUCTION

Efficient connectivity is essential for the fast and faithful delivery of signals across local and distal brain regions. White matter axons with a sufficiently large axon diameter rely on myelin sheets from nearby oligodendrocytes for saltatory conduction of their action potential along the length of their axon. Although neuronal architecture is largely fixed in adult mammals, in contrast, oligodendrocytes remain highly plastic throughout adulthood and oligodendrocyte precursor cells (OPC) are the major proliferating cell types in adult brains, constituting up to 9% of all cells in the white matter (Dawson *et al*. 2003). The continued plasticity into adulthood may be an important factor in continued motor learning and high level cognitive functioning in humans (Wang and Young 2013).

Studies using in vivo diffusion magnetic resonance imaging (MRI) methods have started to give insight into dynamic alterations in white matter after training novel motor tasks such as juggling (Scholz *et al*. 2009). Diffusion MRI as measured with diffusion tensor imaging (DTI) offers a method of measuring integrity and orientation of white matter in vivo (Figure 1). DTI allows for the extraction of four types of measures that are selectively sensitive to alterations in diffusivity that are thought to reflect specific biological processes. Fractional anisotropy (FA) is a summary measure of structural integrity. Mean diffusivity (MD) is an inverse measure of membrane density, and is very similar for both gray and white matter, while being high for cerebral spinal fluid. MD is sensitive to cellularity, edema, and necrosis. Axial diffusivity (AD) tends to be sensitive to pathology, in axonal injury AD decreases. AD of white matter tracts is reported to increase with brain maturation. Radial diffusivity (RD) is thought to increase in white matter during de- or dys-myelination. Despite the low inherent resolution that is common to this modality, DTI offers an exciting new avenue for studying alterations in human brain architecture after learning a new skill. "*Diffusion Tensor Imaging is a cutting edge imaging technique that provides quantitative information with which to visualize and study connectivity and continuity of neural pathways in the central and peripheral nervous systems in vivo.*" (Basser *et al.* 2000). It is however unknown how these changes in DTI measures relate to the actual underlying cellular processes, and if it is a true reflection of structural alterations in white matter due to learning or a more transient cause like blood flow alterations. A first basic exploration of this question was initiated

by Sampaio-Baptista *et al.* (Sampaio-Baptista *et al.* 2013). By using a rodent model they investigated how motor skill learning would alter MRI measures of white matter and how these alterations related to myelin basic protein (MBP) staining. They collected DTI from rats after they trained them on a reaching task. Results showed that rats who were trained on the reach task had significantly lower measures of density and integrity of white matter contralateral to the reaching arm. Furthermore MBP staining indicated that the levels of myelin were increased in the reach group and that these levels correlated with the reach effectiveness. A critique of this study is that they sacrificed the animals before running the MRI scan, and only ran a between subjects analysis.

In a similar study, Sagi *et al.* (Sagi, Tavor, and Assaf 2012) collected DTI scans before and after a spatial learning task. They tested this in both human and rodent subjects and found similar alterations, showing reduced levels of mean diffusivity in the hippocampus. To investigate what cellular processes might underlie these alterations they ran a histology on the rat brains and looked at microtubule associated protein 2 (MAP-2), synaptophysin, glial fibrillary acidic protein (GFAP) and brain derived neurotrophic factor (BDNF). They found significant increases in the levels of synaptophysin, GFAP and BDNF in the animals that were trained on the spatial task, but not the control animals. Indicating that alterations in MD values after training are potentially due to changes in the number of synaptic vesicles and/or glia density.

These types of studies are just beginning to explore the underlying cellular mechanisms that cause change in DTI measures after learning new skills. In this review I will highlight a number of studies that are in support of the thesis that dynamic myelination is necessary for effective skill learning and potentially even complex cognitive functions. I furthermore ask the question how these behaviors are translated and communicated to the oligodendrocytes and their precursors. Specifically, what causes these cells to start dynamically altering migration and differentiation, to cause myelinogenesis of axons in the white matter pathways that ultimately lead to enhanced learning. This review aims to take a closer look at how and when dynamic myelination occurs from early development into adulthood.

	FA	MD $(\lambda_1+\lambda_2+\lambda_3)/3$	AD λ_1	RD $(\lambda_2+\lambda_3)/2$
Gray Matter	↓	–	↓	↑
White Matter	↑	–	↑	↓
CSF	↓	↑	↑	↑
High myelination	↑	↓	–	↓
Dense axonal packing	↑	↓	–	↓
WM Maturation	↑	↓	↑	↓
Axonal degeneration	↓	↑	↓	↑
Demyelination	↓	↑	–	↑
Low SNR	↓	↑	↓	–

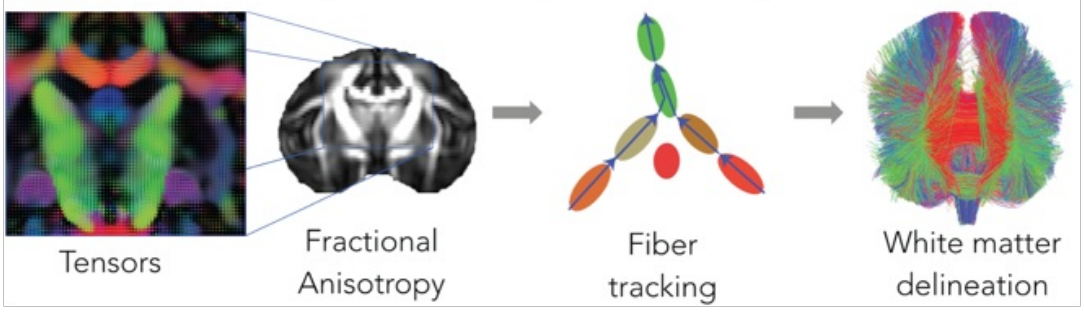

Figure 1: Overview of how alterations in diffusion tensor imaging measures can related to microstructural alterations.

MYELIN DEVELOPMENT AND MAINTENANCE

The uniform and periodic spacing of myelin along the length of axons is dependent on the organized and dynamic distribution of oligodendrocytes and their progenitors. Until a number of years ago it was unknown what determines this spacing, as it could be brought on by signaling cues from axons, or OPCs could have an internal system of determining distribution. In order to understand how OPCs dynamically altered their myelin distribution along target axons, Kirby *et al.* (Kirby *et al.* 2006) used in vivo time lapse confocal fluorescent microscopy to investigate OPCs surrounding axons in the spinal cord of developing zebrafish. They created transgenic zebrafish that would express membrane-tethered fluorescence in oligodendrocyte lineage cells using Nkx2. They found that the remodeling and migration behavior of OPCs is highly variable, their filopodium-like processes often migrate for many hours before myelination occurs. Furthermore, OPCs respond to contact with neighboring OPCs by retracting their filopodia, and will dynamically divide if they find unoccupied spaces in order to migrate to the new location.

Although Kirby at al. showed evidence for the dynamic behavior of OPCs in developing zebrafish spinal cord, it remained unknown if this generalized to the cortex, would continue into adulthood and generalized to mammal species. To answer these questions Hughes *et al.* (Hughes *et al.* 2013) investigated how OPCs in the cortex of adult mice maintain a homeostatic distribution even in the face of sudden cell death. In order to do this the authors developed a mouse line that exhibited fluorescence in cells that expressed NG2+, a marker for OPCs. They then used two-photon imaging through a cranial window to investigate how these cells migrated in vivo. They confirmed previous results, and showed that NG2+ containing OPCs were highly dynamic, had active filopodia that would survey the surrounding area, while avoiding neighboring OPCs. Furthermore, they found that, when existing cells were ablated, NG2+ cells would divide and/or migrate to replace the damaged cells. Together these studies confirmed that OPCs are dynamic from early development into adulthood and can actively respond to injury by replacing damaged cells.

NEURAL ACTIVITY DRIVES MYELINATION

Only a selection of all axons are covered in myelin. Cultured axons in vitro get myelinated when their diameter is bigger than 0.4µm (Hines *et al.* 2015). Is this information sufficient for oligodendrocytes in vivo, or are additional processes at work? A prerequisite for the thesis that myelinating oligodendrocytes are essential for learning, is that neurons and axons are capable of signaling activity levels to neighboring oligodendrocytes and their progenitors. In this seminal paper Demerens *et al.* (Demerens *et al.* 1996) are the first to investigate the role of electric activity on myelin formation. They used both an in vitro and an in vivo model to investigate if blocking or increasing action potentials would alter axon myelination. They show that treating in vitro embryonic mouse brains at 8 DIV for 2, 4 or 6 days with tetrodotoxin (TTX) leads up to 98% reduction in myelinated fibers two weeks later, although this effect disappeared at 18 DIV. Conversely a-scorpion toxin (a-ScTX), which increases the duration and frequency of spontaneous action potentials lead to a 2.4 fold greater number of myelinated segments. To confirm these effects in vivo they injected TTX intravitreously in mice at day P4, after which they examined the optic nerve at P6. It showed a reduction in myelinating oligodendrocytes of 75%, this effect disappeared when TTX was injected at P5. These are the first indications of an interaction between neuron activity and myelination. Although in this case the effect was very sensitive to a specific developmental time period.

Demerens *et al.* (Demerens *et al.* 1996) effectively showed that electric activity promotes axonal myelination in development. What processes are at work to convert action potentials in an axon to increased myelination by oligodendrocytes? Hines *et al.* (Hines *et al.* 2015) used in vivo confocal time-lapse imaging to investigate axons in the spinal cord of developing zebrafish. They were able to label both the axons and the myelin sheets using enhanced green labeled phox2b+-cells and red fluorescent

sox10 +-cells respectively. As seen before, exposure to TTX lead to reduced levels of myelin wrapping, but an indiscriminate increase in action potentials due to veratridine did not alter myelin levels. To test if selective alterations in neural activity would bias myelination they designed a transgenic zebrafish line with a mosaic overexpression of tetanus neurotoxin light chain (TeNT) which inhibited synaptic vesicle exocytosis selectively in phox2b+ axons. They found that after initial sheathing only a proportion (25%) stabilized while the rest were retracted in an activity and vesicle release dependent fashion. These studies show that both electric activity and vesicle release are able to signal myelin producing cells to alter myelinogenesis in response to these signals.

MOTOR LEARNING REQUIRES DYNAMIC MYELINATION

So far we have seen that myelin is dynamically distributed along axons throughout adulthood and that oligodendrocytes can modify their behavior in response to electric activity and vesicle release of axons. These results hint at the possibility that myelin formation can take place in response to specific behavior. Is this however sufficient for learning complex motor behaviors? In this study Gibson *et al.* (Gibson *et al.* 2014), elegantly explore if optogenetic stimulation of the premotor cortex in awake mice would lead to altered levels of myelination in those regions, and if those levels of myelination were associated with improved motor activity in the previously stimulated limb. They aimed to see if they could induce differentiation of oligodendrocyte progenitor cells and directly influence myelinogenesis in adult living mice. To investigate this they used a mouse-line with channelrhodopsin infection of Thy1 expressing neural cells that predominantly are located in layer 4 of the premotor cortex. Placement of the optical fiber just below the pial surface allowed for stimulation of layer 4 without causing damage to those neurons. The mice would exhibit circular walking motions during repeated unilateral stimulation at rates that were comparable to physiological firing rates of projection neurons in this layer. They found that both juvenile and adult mice showed altered OPC production and myelination levels after stimulation as compared to wild type litter mates. The administration of the proliferation marker and thymidine analog 5-ethynyl-2′-deoxyuridine (EdU) after optogenetic activation showed labeled cells in the targeted premotor cortex and in the subcortical white matter of the corpus callosum 3 hours after stimulation, but not 3 weeks after stimulation. Characterization of these proliferating cells showed that 54% labeled positive for Olig2 markers, and 24.5% for PDGF receptor-α. To test if these results were not due to indiscriminant activity they induced a motor seizure which did not replicate the previous results. Furthermore, histone modifications in EdU positive cells were consistent with an alteration in repressive and activating regulatory elements between 3-24 hours after activation. Transmission electron microscopy results confirmed that myelin thickness relative to axon caliber (g-ratio) was decreased, indicating an increase in myelin thickness in the premotor cortex. This was further confirmed with MBP staining in this region. Analysis of movement 4 weeks after neural activation showed that these mice had improved swing speed during normal gait. Indicating a correlation between oligodendrogenesis, myelin thickness and movement quality. However, this improvement in muscle movement could be due to synaptic alterations. To test this hypothesis they used an HDAC inhibitor that would enhance synaptic plasticity, but block oligodendrogenesis. The result showed that there was no altered g-ratio nor was there a behavioral effect after stimulation, supporting the idea that oligodendrocyte differentiation and/or myelination was necessary for the motor improvement after stimulation.

The usage of an HDAC blocker is a rather crude method of inhibiting oligodendrogenesis. To combat this McKenzie *et al.* (Mckenzie *et al.* 2014) use a conditional knock-out of myelin regulatory factor. The authors first show that learning a new motor skill, in this case the complex running wheel, can temporarily upregulate OPC proliferation and differentiation in adulthood. This indicates a correlation between motor learning and OPCs, but it is not sufficient to claim that OPC proliferation and differentiation is necessary for motor learning. To investigate if late born oligodendrocytes can contribute to motor learning this group designed a mouse model where myelin regulatory factor (MyRF) transcription in OPCs was conditionally knocked-out using the Cre-flox technique in cells positive for the OPC-specific marker PDGF receptor-α. Inactivation of MyRF at postnatal day 60 lead to a large reduction in immature oligodendrocytes to about 10% of regular amounts. This reduction will inhibit the

maturation of oligodendrocytes and subsequent axonal myelination. Administration of the proliferation marker EdU, confirmed that MyRF negative mice had fewer new oligodendrocytes. Although this knock-out inhibited the development of new oligodendrocytes, it did not influence the existing number or density of myelinated axons, furthermore new oligodendrocytes were not necessary to recall a previously learned skill. It did however alter learning of the complex running wheel in MyRF negative mice, and it significantly reduced the maximum speed that MyRF negative mice could accomplish in the complex running wheel. Indicating that there is a causal relationship between oligodendrocyte proliferation, myelination and the efficacy at which these adult mice are able to learn a new motor skill.

These studies showed compelling evidence for the requirement of active myelination for motor learning. A question even the previous studies left unanswered is how exactly oligodendrocytes receive the signal to start myelination. De Biase *et al.* (De Biase *et al.* 2011) attempted to answer this questions by looking the glutamate receptor NMDA. Since NMDA receptors are of great importance for neural progenitor differentiation and migration he authors decided to investigate what the role of NMDAR is in OPCs. They specifically explored if an OPC specific deletion of the NMDA receptor subunit NR1 lead to an alteration in OPC proliferation, differentiation and migration in mice. They found that this removal did not alter survival, proliferation, migration or differentiation of OPCs during post-natal development, and myelination was preserved. There was however an increase in the surface expression of AMPA receptors in OPCs.

DISCUSSION

In this overview we looked to see if adaptive axonal myelination is necessary for learning complex motor behaviors. To explore this thesis we first had to investigate if oligodendrocyte precursors are capable of dynamic behavior to ensure effective development and maintenance of myelination from embryo into adulthood. An early study in this field showed that OPCs indeed exhibit dynamic behaviors in response to their surroundings and neighboring OPCs. They do this to ensure an organized distribution of OPCs along the length of the axon. Furthermore, ablation of OPCs lead to alterations in behaviors in OPCs, e.g. migration and differentiation, to ensure the continued coverage of axons with myelin.

Next we investigated if oligodendrocytes and their progenitors can dynamically respond to neuronal activity. Specifically does neural activity drive myelination, and how does it do so. First we saw that inhibition and over-activation of action potentials in neurons lead to altered levels of myelinogenesis. Next we saw that the inhibition of vesicle release cause neurons to retract previously myelinated axons. Together these results indicated that indeed neuronal activity leads to altered myelin levels in vivo, and this is brought on by altered OPC differentiation to cause new oligodendrocytes to myelinate axons in an activity dependent matter.

Finally, we investigated if dynamic myelination is necessary for learning. We first saw that optogenetic stimulation of the premotor cortex lead to altered levels of myelin and improved motor behaviors. When differentiation was blocked with an HDAC inhibitor myelin levels no longer changed, and neither did the motor behaviors improve. In the next study the took this further and saw that knock-out of myelin regulatory factor in precursor cells prevented mice from effectively mastering the complex running wheel. Although there must be a mechanism by which axons signal activity to oligodendrocytes this is not due to NMDA receptor, since inactivating this receptor did not lead to altered levels of myelination. Overall these results indicate that indeed there is compelling evidence that dynamic myelination in adulthood is necessary for effectively learning novel motor skills. Thus lending support to the initial MRI studies that showed alterations in white matter structure after motor learning took place.

Although we do not yet know what the exact signaling mechanism is between neurons and oligodendrocytes, we can speculate. Geurts *et al.* (Geurts *et al.* 2003) investigated white matter of individuals with multiple sclerosis, a disease with profound effects on myelination - specifically de-myelination, and found that metabotropic glutamate receptor expression was altered in affected tissue. According to Gallo and Ghiani (Gallo and Ghiani 2000) a potential role for both metabotropic and

ionotropic glutamate receptors in glia includes regulation of proliferation and differentiation. While others (Maldonado and Angulo 2014)suggest a role for GABA signaling.

In conclusion, the importance of adaptive myelination of specific white matter pathways in response to learning behaviors has given us a new appreciation for the role of oligodendrocytes and their progenitors. Although the exact mechanism of communication between active neurons and oligodendrocytes is yet unknown, this is yet another example of the versatility and importance of glia cells in the brain.

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