From DEPARTMENT OF CELL AND MOLECULAR BIOLOGY
Karolinska Institutet, Stockholm, Sweden

BRAIN MATTER MATTERS: RENEWAL IN THE HUMAN BRAIN

Maggie Yeung Wong

Stockholm 2017

Cover image shows an illustration of human brain white matter cells − the oligodendrocytes are old (tree trunk), whereas their myelin is young (leaves). This was revealed by analysis of ^{14}C from nuclear bomb tests, shedding light on how these cells can contribute to brain plasticity. Photo by Joel Foo and illustration by Mattias Karlén.

Published by Karolinska Institutet.
Printed by Eprint AB 2017

ISBN 978-91-7676-734-4

BRAIN MATTER MATTERS:
RENEWAL IN THE HUMAN BRAIN

THESIS FOR DOCTORAL DEGREE (Ph.D.)

The public defence of this thesis will take place, in lecture hall Rockefeller, Karolinska Institutet, Nobels väg 11, Solna on Friday 16th of June, 2017, at 9:30 AM.

Maggie Yeung Wong

Principal Supervisor:
Prof. Jonas Frisén
Karolinska Institutet
Department of Cell and Molecular Biology

Co-supervisor(s):
Dr. Kirsty Spalding
Karolinska Invstitutet
Department of Cell and Molecular Biology

Associate Prof. Marie Carlén
Karolinska Institutet
Department of Neuroscience

Opponent:
Prof. Klaus-Armin Nave
Max Planck Institute for Experimental Medicine
Department of Neurogenetics

Examination Board:
Associate Prof. Gonçalo Castelo-Branco
Karolinska Institutet
Department of Medical Biochemistry and Biophysics

Prof. Torkel Klingberg
Karolinska Institutet
Department of Neuroscience

Prof. Christos Samakovlis
Stockholm University
Department of Molecular Biosciences,
The Wenner-Gren Institute

Institutionen för Cell – och Molekylärbiologi,
Karolinska Institutet

Brain matter matters: Renewal in the human brain

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i föreläsningssalen Rockefeller, Nobels väg 11, Karolinska Institutet Campus Solna.

Fredagen den 16 juni, 2017, kl 09.30

av

Maggie Yeung Wong

Huvudhandledare:
Prof. Jonas Frisén
Karolinska Institutet
Institutionen för Cell - och Molekylärbiologi

Bihandledare:
Dr. Kirsty Spalding
Karolinska Institutet
Institutionen för Cell - och Molekylärbiologi

Docent Marie Carlén
Karolinska Institutet
Institutionen för Neurovetenskap

Fakultetsopponent:
Prof. Klaus-Armin Nave
Max Planck Institute for
Experimental Medicine
Department of Neurogenetics

Betygsnämnd:
Docent Gonçalo Castelo-Branco
Karolinska Institutet
Institutionen för Medicinsk Biokemi och Biofysik

Prof. Torkel Klingberg
Karolinska Institutet
Institutionen för Neurovetenskap

Prof. Christos Samakovlis
Stockholms universitet
Institutionen för Molekylär Biovetenskap
Wenner-Grens institut

Stockholm 2017

Anyone who stops learning is old, whether at twenty or eighty. Anyone who keeps learning stays young.

− Henry Ford

ABSTRACT

Many cells have a shorter life span than the organism itself and are continuously being replaced. However, for many cell types in the human body it is still largely unknown whether they turn over at all once development is complete. The work presented in this thesis focuses on understanding the occurrence of cell renewal in the human brain and how it may affect brain function. We further explore if the ability of cell renewal may also be affected in the diseased brain. To investigate cell turnover in the human brain we used a methodology developed in the Frisén laboratory. The developed strategy takes the advantage of the integration of the ^{14}C (carbon-14) isotope, produced from the nuclear bomb tests during the Cold War, into genomic DNA, to determine the age of cells in humans.

In **Paper I** we assessed the dynamics of oligodendrocyte generation and myelination in the human brain. We found that the number of oligodendrocytes in the corpus callosum is established in childhood and remains constant thereafter. We revealed that myelin is continually exchanged, whereas white matter oligodendrocytes are remarkably stable with an annual exchange of 1 in 300 oligodendrocytes. This suggests that myelin modulation in humans may be carried out by mature oligodendrocytes. Moreover, we also found different kinetics of oligodendrocyte generation and turnover between gray and white matter, with a longer period of oligodendrocyte generation and higher turnover rate throughout life in gray matter, showing the possibility of de novo myelination in the sparsely myelinated cortex. However, how generation kinetics of the oligodendrocyte population may change under pathological conditions such as in the demyelination disease multiple sclerosis has been unknown.

In **Paper II** we investigated the oligodendrocyte generation dynamics in multiple sclerosis patients. We observed no induced generation of oligodendrocytes in normal appearing white matter, except in a subsets of multiple sclerosis patients with a very aggressive progression of the disease. We also revealed that oligodendrocytes in remyelination lesions were old in multiple sclerosis patients, showing that existing and not new oligodendrocytes are regenerating myelin in multiple sclerosis.

In **Paper III** we examined the extent of postnatal olfactory bulb neurogenesis in humans. We report that the olfactory bulb neurons are almost as old as the individuals, establishing that there is very limited, if any, postnatal neurogenesis in the human olfactory bulb.

Together the findings presented in this thesis highlight a conceptual and fundamental difference in human brain plasticity compared to other mammals.

LIST OF SCIENTIFIC PAPERS

I. **Yeung M.S**., Zdunek S., Bergmann O., Bernard S., Salehpour M., Alkass K., Perl S., Tisdale J., Possnert G., Brundin L., Druid H., Frisén J. (2014). Dynamics of oligodendrocyte generation and myelination in the human brain. Cell *159*, 766-774.

II. **Yeung M.S**., Djelloul M., Steiner E., Bernard S., Salehpour M., Possnert G., Brundin L., Frisén J.
Remyelination by old oligodendrocytes in multiple sclerosis.
Manuscript

III. Bergmann O., Liebl J., Bernard S., Alkass K., **Yeung, M.S**., Steier P., Kutschera W., Johnson L., Landen M., Druid H., Spalding K., Frisén J. (2012). The age of olfactory bulb neurons in humans. Neuron *74*, 634-639.

Publications not included in this thesis:

Djelloul M., Holmqvist S., Boza-Serrano A., Azevedo C., **Yeung M.S.**, Goldwurm S., Frisén J., Deierborg T., Roybon, L. (2015). Alpha-Synuclein Expression in the Oligodendrocyte Lineage: an In Vitro and In Vivo Study Using Rodent and Human Models. Stem Cell Reports *5*, 174-184.

Forsberg M., Carlén M., Meletis K., **Yeung M.S.**, Barnabé-Heider F., Persson M.A., Aarum J., Frisén J. (2010). Efficient reprogramming of adult neural stem cells to monocytes by ectopic expression of a single gene. Proceedings of the National Academy of Sciences *107*, 14657-14661

Carlén M., Meletis K., Göritz C., Darsalia V., Evergren E., Tanigaki K., Amendola M., Barnabé-Heider F., **Yeung M.S.**, Naldini L., Honjo T., Kokaia Z., Shupliakov O., Cassidy R.M., Lindvall O., Frisén J. (2009). Forebrain ependymal cells are Notch-dependent and generate neuroblasts and astrocytes after stroke. Nature Neuroscience *12*, 259-267.

CONTENTS

LIST OF ABBREVIATIONS

^{12}C, ^{13}C, ^{14}C	carbon isotopes, ^{12}C, ^{13}C, ^{14}C
^{3}H	tritiated thymidine
AMS	accelerator mass spectrometry
APC	adenomatous polyposis coli
BrdU	5-bromo-2'-deoxyuridine
CNPase	2',3'-cyclic nucleotide 3'-phosphodiesterase
CNS	central nervous system
CC-1	APC monoclonal antibody, clone CC-1
DNA	deoxyribonucleic acid
ERK 1/2	extracellular signal-regulated kinase 1 and 2
FA	fractional anisotropy
FACS	fluorescent activated cell sorting
G1, S, G2, M	cell-cycle phases: gap 1, synthesis, gap 2, mitosis
IdU	iododeoxyuridine
ITPR2	inositol 1,4,5-triphosphate receptor 2
MBP	myelin basic protein
MS	multiple sclerosis
NFOL1,2	newly formed oligodendrocytes 1 and 2
NOGO-A	neurite outgrowth inhibitor, isoform A
OPC	oligodendrocyte progenitor cell
PCNA	proliferating cell nuclear antigen
PHH3	phosphohistone-H3
PTEN	phosphate and tensin homolog
qRT-PCR	quantitative reverse transcription polymerase chain reaction
QKI-7	quaking-7
RNA	ribonucleic acid
RMS	rostral migratory stream
SOX10	sex determining region Y-box 10

1 INTRODUCTION

Many cells have a shorter life span than the organism itself. Moreover, the cells are continuously being replaced, thus enabling tissue homeostasis, the maintenance of the normal tissue function and morphology of the organism. One essential mechanism of maintaining the function and form in developed tissue is the process of cell turnover. This process is strictly regulated as it prevents tissues and organs from atrophy or tumor development. Simply stated, cellular turnover is a process where certain differentiated cells are eliminated regularly and exchanged typically through division of adult stem cells and their progeny. A lot of research has provided great insights into cell generation and cell turnover in humans, such as in the hematopoietic system, the skin and intestine. However, for many cell types, it is still largely unknown if new cells are generated and replaced once development is complete.

How to measure the life span and turnover rate of certain cell types in the human body is still an unresolved question among scientists. For many organs such as the brain, it remains unknown how often particular cells are replaced in different tissue types and how old different cell types can get. The answers are pivotal as the life span of certain cell types is directly related to the degeneration of physiological function, aging and disease, and subsequently to the potential for cellular regeneration in that particular organ.

The brain combines remarkable stability that permits long-time storage of memories with plasticity, which enables adaptation to new conditions and the constant formation of new memories. During the last years one type of plasticity has gained attention – the generation of new cells. Both neuronal and glial cell generation contribute to neural plasticity and to some degree neural repair. Now, we know that new neurons are continually added in the adult brain of all mammals studied. Moreover, increasing evidence over the past few years has emerged that the generation of the glial cells, the oligodendrocytes in the adult brain, is much more widespread than neurogenesis and that they also may influence neural plasticity. However, substantial differences between mammalian species have been discovered regarding the extent of, as well as the mechanisms behind cell renewal in neural plasticity. It is thus of great importance to study these processes in humans as it is difficult to know how well the information from animal models could be translated into the human situation. This thesis aims to increase our understanding of cell generation in the human brain, and in particular the generation of oligodendrocytes and their myelin and the role they may play in neuronal function modulation.

2 CELL RENEWAL IN THE CENTRAL NERVOUS SYSTEM

2.1 HOW CAN WE IDENTIFY NEWLY GENERATED CELLS IN HUMANS?

Most of our knowledge regarding cell turnover and proliferation potential in the adult human body comes from studies in nonhuman species and in particular rodents. Many of these studies rely either on techniques involving the detection of molecular markers of cell division or a labeling paradigm which allows for visualization of newborn cells by incorporation of modified nucleotide into the DNA of dividing cells. In the following two sections I will highlight some of the techniques commonly used to study cell generation. Though these methods have been instrumental in revealing cell generation in experimental animals, they bear several limitations when investigating the life cycle of human cells and in particular those with a slow turnover rate. In the last section (2.1.3), a different strategy is presented, not associated with these kinds of limitations, which was used in Paper I, II, and III.

2.1.1 Proliferation markers

To assess cell generation, traditional methods often involve the detection of molecular markers present in the different stages of the cell cycle when new cells are generated. Commonly used markers for proliferations are: 1) Ki-67, which is present throughout all active phases of the cell cycle (G1, S, G2 and M) (Scholzen and Gerdes, 2000): 2) proliferating cell nuclear antigen (PCNA), a cofactor of DNA polymerase that encircles the DNA and thus present in the stage of when DNA synthesis (S-phase) takes place (Moldovan et al., 2007) and 3) phospho-histone H3 (PHH3), where histone-H3 gets phosphorylated during late G2 phase and mitosis (M-phase) (Hans and Dimitrov, 2001) Analysis using these cell division or mitotic markers is very useful in identifying the proliferative state of cell populations in the tissue and when comparing cell proliferation in different tissue types and conditions. Although it gives a good view of the numbers of cells with mitotic activity, it is also limited, as it provides a static view at only one single time point and thereby cannot provide any insight into the future fate of the labeled cell. Information whether the progeny will mature and integrate into the tissue and, if so, to what extent, is not conclusive. In addition, these cell proliferation markers are not able to give any information on the past history of the cells and are thereby unable to determine when the cells were born.

2.1.2 Prospective labeling paradigms – nucleotide analogues

Another strategy to examine the generation of new cells is by prospective labeling of dividing cells and tracing the progeny. This prospective labeling paradigm involves the administration of labeled nucleotides analogues, such as the historically used radioactive ^{3}H - thymidine (Altman, 1962) the more commonly used 5-bromo-2'-deoxyuridine (BrdU) (Gratzner, 1982) and similar halogenated uridines or the more recent 5-ethynyl-2'-deoxyuridince (EdU) (Salic and Mitchison, 2008). These synthetic thymidine analogues integrate stably into the DNA during the S-phase of the cell cycle when new DNA is synthesized, thereby allowing the labeling of newborn cells and their progeny. These labels can be visualized in the tissue section and in combination with cell type-specific markers and thereby reveal the generation and integration of mature cells in the tissue and organ.

These prospective labeling paradigms have been instrumental in revealing cell generation in experimental animals – however, there are some limitations. For example, cells undergoing DNA repair or going through cell death have been reported to incorporate labeled nucleotides into the DNA and can therefore falsely give the impression that the cells are newly generated (Taupin, 2007). Moreover, the labeled nucleotides may interfere with the investigated process, as it can be toxic when used in higher concentration or administered for longer time periods and can result in the underestimation of cell generation (Ross et al., 2008; Young et al., 2013). The ability of labeled nucleotides to stably integrate into the DNA makes them potentially toxic, mutagenic and carcinogenic and consequently not applicable for use in healthy humans. In unusual cases, labeled nucleotides have been administered to cancer patient as a diagnostic tool or for radiosensitization and the post-mortem tissues could be obtained for analysis of cell generation in the adult human brain (Bhardwaj et al., 2006; Eriksson et al., 1998, Paper I). However, in these studies many of the patients received the labeled nucleotide shortly before death, making detection of cell types with low generation rate challenging. Furthermore, one needs to be reminded that many of the cancer patients were also treated with radiotherapy or chemotherapy, making it difficult to extrapolate the data to healthy individuals.

2.1.3 Retrospective birth dating using ^{14}C

Carbon-14, (^{14}C) is the only radioactive isotope and the least abundant of the three naturally occurring carbon isotopes (^{12}C, ^{13}C, ^{14}C, 99%, 1% and 1 part per trillion of all carbon, respectively). ^{14}C is naturally generated by interaction of cosmic ray with nitrogen in the atmosphere. The generated ^{14}C further reacts with oxygen and forms $^{14}CO_2$ in the atmosphere. Historically, the atmospheric level of ^{14}C has been very low and constant (Figure 1A) until the Cold War (1955-1963) (Levin and Kromer, 2004;

Nydal and Lövseth, 1965). The extensive above ground nuclear bomb tests during this period dramatically increased the ^{14}C levels in the atmosphere, reaching its peak in 1964 (Figure 1C). As a result of the Partial Test Ban Treaty that was signed in 1963, the levels have progressively decreased since then. This decline is not due to the radioactive decay (as ^{14}C half-life is 5730 years) of ^{14}C, but mainly attributed to diffusion from the atmosphere into the oceans and the biotope. The $^{14}CO_2$ in the atmosphere enters the biotope through photosynthesis and is subsequently transferred into humans through the food chain. Consequently, the content of ^{14}C in biomaterial could be reflected in the atmospheric concentration at the time of synthesis. Measurements of ^{14}C in tree rings further confirmed this fast transfer of ^{14}C from the atmosphere to living organisms (Figure 1B,C).

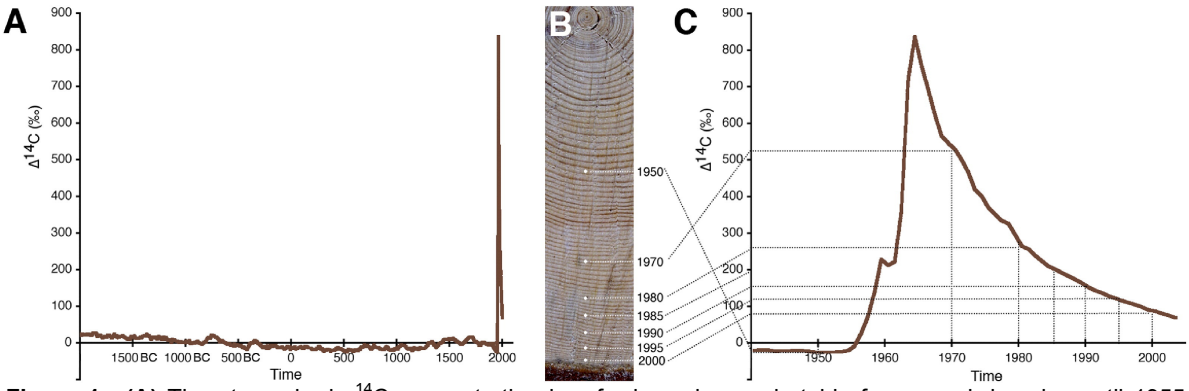

Figure 1. **(A)** The atmospheric ^{14}C concentration has for been low and stable for several decades until 1955 when it dramatically increase due to the nuclear bomb tests during the cold war. **(B, C)** Measurements from tree rings confirm the fast ^{14}C transfer from the atmosphere into the biotope. **(C)** Magnification of the increase in (A). Spalding et al., 2005. Reprinted with permission from publisher.

DNA is a very stable biomolecule and the carbon in genomic DNA is principally exchanged only during cell division. Carbon contribution from DNA methylation and post-mitotic nucleotide exchange in cells, such as DNA repair, is minimal and below detection limit (Bergmann et al., 2012; Ernst et al., 2014; Spalding et al., 2005). This was shown even in stroke cases when DNA repair is increased and carbon influence from these sources is largely negligible (Huttner et al., 2014). The ^{14}C levels integrated into genomic DNA mirror the levels in the atmosphere at any given time and can thus serve as a date mark for when cells are born. Taking advantage of this, we have developed a strategy to retrospectively date certain cells in the human brain (Paper I, II and III) by measuring the genomic DNA ^{14}C levels from human post mortem tissue. This strategy is further referred to as the ^{14}C birth dating technique.

2.1.3.1 Establishing the birth of cells

To establish the age of a cell population, the proportion of ^{14}C in genomic DNA is measured with highly sensitive Accelerator Mass Spectrometry (AMS). The sensitivity of the AMS methodology has been further developed by Salehpour and colleagues (Salehpour et al., 2008; Salehpour et al., 2013) and it now allows the measurement of biological samples in the range of micrograms of carbon. However,

it will not be possible to reach a single cell resolution due to that ^{14}C still is a very rare isotope, with an average of 1 ^{14}C atom in the DNA of approximately every 15th cell in the human body.

Once the ^{14}C concentration has been measured, the resulted value is reported as $\Delta^{14}C$ over time and compared to the atmospheric ^{14}C concentration (Figure 2). The observed negative atmospheric ^{14}C level during certain periods (Figure 1) is due to $\Delta^{14}C$ being a relative measurement unit normalized to a standard and corrected for decay and isotope fractionation.

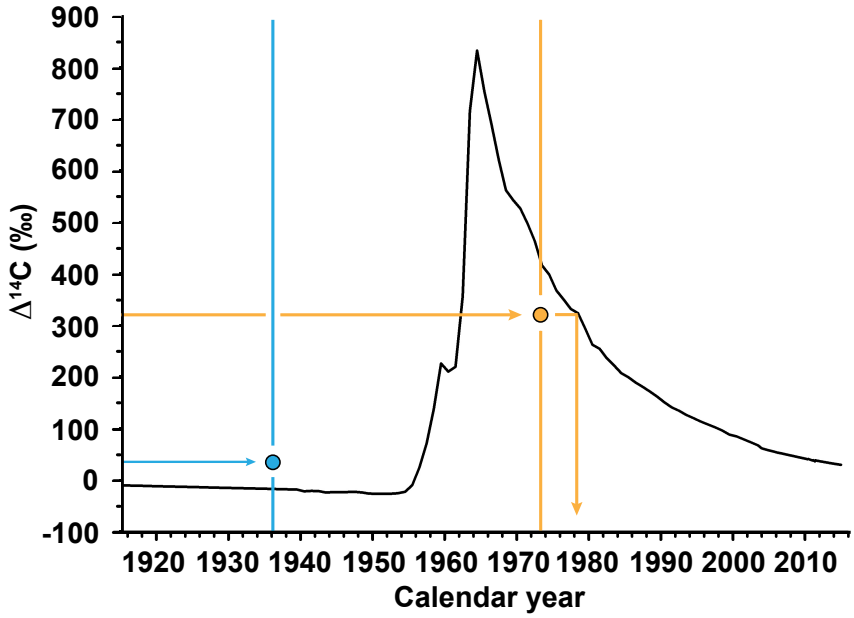

Figure 2. Schematic picture depicting the strategy of establishing the birth date of cells. The curve shows the atmospheric ^{14}C concentration over time. The hypothetical data points (circles) are placed correspondingly to the date of birth on the individual (vertical lines) and the ^{14}C concentration in genomic DNA. For an individual born after the nuclear bomb tests (1955-63), the date of birth of the cell population can be directly inferred from the x-axis, whereas this is not the case for an individual born before the onset of the increase in atmospheric ^{14}C.

The data point of the measured ^{14}C concentration of the cell population (circle) is placed on the date of birth of the subject. For subjects born after the nuclear bomb test (1955-63), the average birth date of the cell population can be directly inferred by the intersection with the atmospheric ^{14}C levels and then read off in the x-axis (year). Whereas for subjects born before the increase in ^{14}C it is not possible to infer the time of birth of the cell population as there may be two intersections of the atmospheric ^{14}C curve and consequently two potential birth dates (Figure 2). However, the measured values from these subjects give a good indication of any turnover level (incorporation of later generated ^{14}C), as large deviation from the atmospheric ^{14}C curve indicates high turnover and values on the curve indicate no turnover.

The detection sensitivity of cellular turnover evidently depends on the number of individuals analyzed but more specifically on when the individual was born in comparison to when the new cells were generated. For a person born just before the vast increase of the atmospheric concentration of ^{14}C, the highest relative difference in ^{14}C is achieved between the time of birth and the period of the nuclear bomb test and the decrease just thereafter. This provides the highest sensitivity for detecting cell generation that may have occurred postnatally (during the childhood period). For an individual born around 20 years before the nuclear bomb test, the period of the highly elevated levels will instead overlap with the time period when the individual reaches adolescence and early adulthood and therefore can provide the highest sensitivity for detection of cellular turnover during this period of life. In contrast, the analysis of a cell population from an individual born after the nuclear bomb test, will give the highest sensitivity for the latest generated cells. Hence, analysis of several individuals of different ages and of different birth dates in relation to the nuclear bomb test will provide the highest total sensitivity of cell turnover detection and provide information on when a cell population is generated in time. It will also enable the detection of relatively small addition of new cells (Bergmann et al., 2009; Spalding et al., 2013, Paper I, II).

To be able to assess any generation dynamics of a cell population, such as turnover rates, mathematical modeling is required. With mathematical modeling, the cell birth and death rate of cell populations can be calculated, determined by the different ^{14}C levels in the cell population. Cell turnover modeling consists of testing different scenarios to find the rates that can best reproduce the measured ^{14}C levels (Ernst et al., 2014; Spalding et al., 2008; Spalding et al., 2013, Paper I, II, III). Our method of ^{14}C birth dating and subsequent mathematical modeling has become a powerful tool in examining cellular turnover in the healthy and pathological human brain (Paper I, II, III). This is mainly due to the advantage of the technique in detecting cell turnover retrospectively without exposing the individual to any chemicals or compounds, as well as, providing cumulative information about the total amount of cellular turnover that may have occurred over the entire lifespan of the individual.

2.2 WHITE MATTER RENEWAL

2.2.1 Myelin: Not just brain glue – 300 years of myelin history

Around half of the human brain is composed of white matter which makes up a disproportionally larger proportion of the prefrontal cortex than with nonhuman primates (Schoenemann et al., 2005). White matter refers to the brain region underlying the gray matter cortex and consists of tightly packed nerve fibers coated with the lipid-rich electrical insulation called myelin, giving rise to the white appearance. As the nervous system grew more complex, the development of the lipid-rich wrap around the axons of neurons was a fundamental step in overcoming the pressure of expanding the size of the nervous system in order to get faster conduction velocities, thereby enabling the emergence of more complex behavior (Salzer and Zalc, 2016). In addition to facilitating the fast conduction of nerve impulses, myelinating cells provide also metabolic and trophic support of the axonal integrity (Nave, 2010).

In the beginning of the 18th century, the first scientist to observe and describe myelinated fiber was most likely Antonie van Leeuwenhoek, who describes his findings in a letter to the London Royal Society, *Philosophical Transactions of the Royal Society* (Schierbeek, 1953). Another century passed before Christian Ehrenberg (1833) described myelinated nerve cells as cylindrical tubes surrounded with medulla, white matter (Boullerne, 2016). Five years later, Robert Remark distinguished the surrounding medulla from the main part of the nerve cell but not as it is known today as the “axon” and “myelin” (Boullerne, 2016).

Almost 150 years after the first observation, the term myelin was first coined by Rudolf Virchow. Rudolf Virchow thought that since myelin at the time was considered to be within the nerve fiber, he likened the medullary substance to the marrow of bones and thus named it “myelin”, which is derived from the Greek “myelos”, marrow (Virchow, 1863). Rudolf Virchow also observed other cells than neurons within the connective tissue, the “nervenkitt” (nerve glue), *i.e.* neuroglia, but due to the limitation of the staining methods they remained obscured. At that time it was assumed that myelin was secreted by neurons, however, decades later Pío del Río-Hortega, with his improved histological staining, proposed that myelin may be generated by oligodendrocytes (Iglesias-Rozas and Garrosa, 2012) and this was further reinforced by Wilder Penfield (Penfield, 1924). The role of oligodendrocytes in myelin formation was supported by the observations of their abundance in the white matter tract, their morphology at the time of myelination and their close proximity to myelin sheaths. However, is was not until the development of the electron microscopy, with the seminal work by Betty Ben Geren and later by Mary and Richard Bunge, which established that myelin was not axon derived but a continuous plasma membrane extension of Schwann cells (glial cells in the peripheral nervous system) (Geren, 1954) and oligodendrocytes (glial cells in the central nervous

system, CNS) (Bunge et al., 1962). Together these studies revealed that neurons and their axons are not the source of generating and secreting myelin, which had long been assumed. What had previously been seen as just mere gluey substance part of the neuron and a static insulator, our knowledge of myelin has now improved dramatically, revealing that oligodendrocytes and myelin are much more dynamic than previously thought. In the following section, a short overview will be presented of the emerging field of oligodendrocytes and myelin and how it may influence the adult nervous system.

2.2.2 Does myelin function affect signal conduction?

In the CNS, myelination refers to the process of ensheathment of the axons by the specialized plasma membrane of oligodendrocytes that in a spiral wrapping fashion form compact myelin segments called internodes. Internodal segments are alternated with nodes of Ranvier, which are unmyelinated axonal plasma membrane segments accumulated with voltage-dependent sodium channels that enable saltatory action potential propagation (conduction of nerve impulses) (Tasaki, 1939). The wrapping of the multilayered myelin membrane insulates the internodal regions, which reduces membrane capacitance and increases membrane resistance, thus facilitating the fast saltatory propagation of action potentials (Hartline and Colman, 2007). However, can changes in this myelin structure affect the conduction velocity?

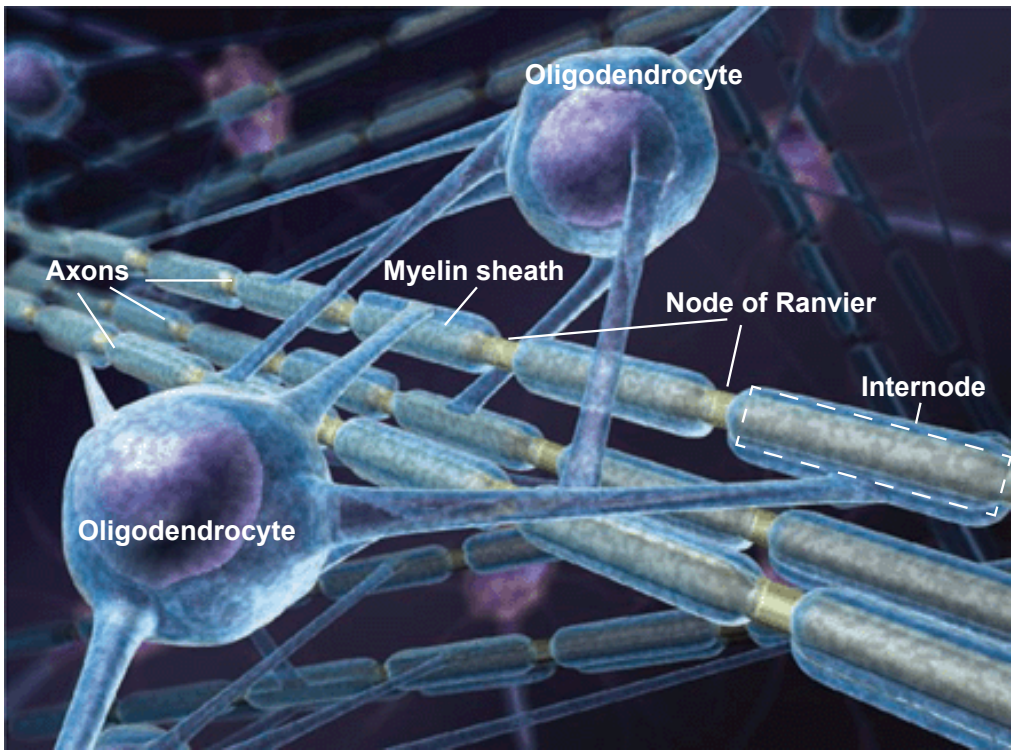

Figure 3. Myelin structure and domains along myelinated axons in the CNS. Adapted from (Fields, 2010). Reprinted with permission from publisher.

How myelin affects the conduction speed depends on the thickness of the myelin sheath in relation to the axon diameter as well as on the internodal length. For example, it has been shown in frogs and mice that increase in individual internode (myelin sheath) length can increase conduction velocity (Huxley and Stämpeli, 1949; Wu et al., 2012). Furthermore, computational models have indicated that even subtle changes in the myelin sheath thickness, relative to the axon diameter, can have profound effects on conduction velocity (Smith and Koles, 1970). This potential of affecting conduction speed suggests that changing these parameters could alter neuronal circuit function.

Most myelinated axons have developed sheaths with a length and thickness close to the calculated optimum for maximal conduction velocity after development. However, there may be neurons in certain areas that not only need to conduct as fast as possible, but also need to synchronize conduction speed, such as to help couple activity of different neurons with different axonal length to discharge synchronously. Thus, a way to enable this would be by affecting myelin thickness and/or internodal length. The integration of auditory input from both ears is a well-described example showing that conduction time can affect function (Seidl, 2014). Simply described, in avians, auditory information is transferred from the ears to neurons in the cochlear nucleus (an area involved in sound processing) on both sides of the brainstem. This in turn further extends axons that divide into two branches, one projecting branch to the ipsilateral region of detector neurons and another much longer branch projection to equivalent neurons on the contralateral side. In order to accurately process the sound information, the signals from both ears to the detector neurons need to arrive simultaneously after signal propagation along the long and the short projection axons from neurons of the cochlear nucleus. To achieve this, anatomical analysis has shown that the longer axon projection has both an enlarged axon diameter and an increased myelin sheath length and thickness (Seidl, 2014; Seidl et al., 2010). Moreover, it has also been observed that the length of internodal segments changes over the lifespan (Young et al., 2013) and depending on the regional location, it can also differ along a single axon (Tomassy et al., 2014). This illustrates the potential capacity of the nervous system to alter the conduction velocity and thus affect the circuit function by fine-tuning myelination.

2.2.3 Myelination of the brain

Myelination in humans is a protracted process which occurs relatively late in development compared with other primates (Miller et al., 2012; Sakai et al., 2011). It is mainly a postnatal process that continues well into adulthood and even into the fourth decade of human life (Lebel et al., 2012; Yakovlev and Lecours, 1967). From histological and neuroimaging studies myelination process has been observed to proceed in a defined spatial and temporal pattern, with most of the spinal cord being

myelinated during early childhood (age 2) and progressing from proximal to distal regions in the brain, with prefrontal and association regions being myelinated last. Generally, less complex brain regions dedicated to basic neurological and homeostatic functions are myelinated earlier than more complex regions associated with high-level cognitive and executive functions. Thus, a region such as the brainstem is myelinated prior to basal ganglia as well as to the cortical association fiber tracts (Brody et al., 1987; Deoni et al., 2011; Lebel et al., 2012; Yakovlev and Lecours, 1967). Moreover, observations show that the axons with larger diameter seem to be myelinated before those with a smaller one (Hildebrand et al., 1993). Similarly, regions myelinated earlier become on average more heavily myelinated than regions that are myelinated later (Glasser and Essen, 2011). What is the significance of this prolonged myelination process? If the myelin function is merely a question of insulation, should this process not already be completed during childhood? Could this prolonged myelination observed in humans be related to learning and cognition? If so, how is myelin reshaped during brain maturation and in response to environmental stimuli?

2.2.4 Experience affects white matter

2.2.4.1 Experience-induced white matter changes during development

Due to prolonged myelination in humans and the overlap with the periods of brain development of certain behaviors and cognitive function, it had been speculated that myelination plays a role in modulating network activity and behavioral performance and that external stimuli (experience) may regulate this process (Fields, 2008). Indeed, several studies have proposed that white matter structure can be influenced by environmental experience. Children that suffered severe neglect during childhood display a reduction in the corpus callosum area (major white matter tract) (Mehta et al., 2009; Teicher et al., 2004). In social isolation studies, rhesus monkeys presented a reduction in corpus callosum size, an effect that lasted at least six months after social rehousing. Moreover, monkeys socially isolated during the juvenile period displayed cognitive deficits in learning tasks (Sánchez et al., 1998). Likewise, social depravation for 2 weeks in developing mice during the critical period (immediately after weaning, postnatal day 21, P21) resulted in a dramatic reduction of myelination in the prefrontal cortex (Makinodan et al., 2012). Alterations in oligodendrocyte morphology, such as fewer and thinner myelin sheaths were observed. The effects on myelination did not improved or recover even after reintroduction of the affected mice to a social environment (Makinodan et al., 2012). Similar changes were also observed in the prefrontal cortex in socially isolated adult mice. However, no-long-lasting effects on myelination were observed and social reintroduction was sufficient to normalize the observed effects (Liu et al., 2012). In a sensory deprivation study in developing mice, the unilateral trimming of the whiskers after birth revealed a

decrease in myelination in the barrel cortex (whisker somatosensory cortex) (Barrera et al., 2013). Remarkably enough, the critical period took place much later in this region, which is consistent with the observation that active myelination in this cortical region occurs later (between P45 and P60) and unlike social isolation during the critical period in juvenile mice, the myelination in barrel cortex was able to recover after the whiskers were allowed to grow again (Barrera et al., 2013). Together these observations suggest that the effects on myelin development caused by experience may vary between different brain regions. Interestingly, in these rodent studies, it is primarily the myelination that is affected and reduced, since the number of oligodendrocytes is normal and unaffected.

2.2.4.2 Does experience affect white matter in the adult?

Through recent advancement human neuroimaging studies have further revealed the impact of experience on white matter microstructure, such as, children who were poor readers displayed lower fractional anisotropy (a measurement of water diffusivity in diffusion tensor imaging, which correlates with myelination, axon fiber density and axonal diameter, FA) implicating reduced myelination in the cerebral white matter compared to good readers. However, after 100 hours of intensive remedial reading instruction, increased FA in the region was observed, suggesting that increased use of the brain area involved in reading correlated with an increase in white matter structure organization (likely myelination) (Keller and Just, 2009). Comparable observations have been seen in studies examining the effect of new motor skill learning, such as long-term piano practicing during childhood and adolescence (Bengtsson et al., 2005). The mammalian CNS myelin continues to be sensitive to experience-influenced structural change even after development. This was seen in adult individuals, who after 6 weeks of intensive juggle learning and training showed increases in FA of white matter regions important for visuomotor control (Scholz et al., 2009). Similar observations of region-specific increases in white matter were also seen in adult subjects after 11 hours meditation practice over a one-month period (Tang et al., 2010). In a rodent study, where adult rats learning a complex reaching skill resulted in FA increase in white matter tract subjacent to the contralateral motor cortex involved in the task but not in the untrained ipsilateral hemisphere. Both the FA and myelin staining (myelin basic protein, MBP) intensity correlated with the learning performance of the animals (Sampaio-Baptista et al., 2013). Rats trained in a spatial learning task also showed similar white matter changes (Blumenfeld-Katzir et al., 2011). Taken together, this suggests that white matter myelin modulation continues well after development has finished but also that experience and neuronal activity may regulate this process.

Consistent with the findings in development and adults, environmental stimuli elicit neuronal activity changes, which in turn may stimulate myelination. Gibson and colleagues tested this idea in vivo by increasing neuronal activity generated by

optogenetic stimulation in the premotor cortex (Gibson et al., 2014). This resulted in increased generation of new mature oligodendrocytes, increased MBP (a myelin sheath protein) protein expression and thickening of myelin sheath within the active circuit, as well as functional improvement of motor function in the mice (increased swing speed of the forelimb) (Gibson et al., 2014). Though neuronal activity in premotor circuit resulted in increased generation of new oligodendrocytes and thickness of myelin sheaths, it is, however, not obvious if the observed myelin modulation is due to the contribution from newly formed oligodendrocytes generating new myelin sheath or if it could be a result of preexisting oligodendrocytes triggered by increased axonal activity and subsequently generate additional myelin wraps to generate the increased myelin thickness observed. As one mechanism does not exclude the other, it would be interesting to see future studies addressing this issue, by for example strategies that enable visualization and separation of preexisting myelin sheath with newly added myelin sheath and myelin from a newly generated oligodendrocyte.

2.2.5 Oligodendrocytes in the adult brain

The oligodendrocytes (specialized glial cell) in the CNS are mostly generated during early postnatal life from oligodendrocyte progenitor cells (OPCs) that have migrated and colonized the gray and white matter (Kessaris et al., 2006). Most of the OPCs divide and differentiate to form mature myelinating oligodendrocytes, however a small proportion of OPCs (2–9% of total cells in the brain) remain proliferative and widely distributed within the adult mammalian brain (Dawson et al., 2003). These OPCs also seem to be present in the adult human brain (Chang et al., 2000). The impact of experience on myelination in the adult brain may potentially result from these OPCs. In the adult rodent brain, the numbers of OPCs remains relatively constant and several genetic lineage-tracing studies in mice have revealed that the OPCs divide, thus continuously giving rise to new myelinating oligodendrocytes throughout adulthood (Dimou et al., 2008; Kang et al., 2010; Rivers et al., 2008; Zhu et al., 2011). In an additional study by Young and colleagues, they showed that in the adult rodent brain there is a continuous generation of oligodendrocytes and formations of myelin sheaths (Young et al., 2013). They observed that around 30% of all mature oligodendrocytes in the corpus callosum and ~6.5% of all mature oligodendrocytes in the optic nerve (fully myelinated white matter tract) were newly formed and there was an age related decrease of internode length, suggesting that active myelination occurs in the fully developed CNS and that the generation of oligodendrocytes contributes to myelin remodeling (Young et al., 2013). However, so far it has remained unknown how this process appears in the human brain.

2.2.6 Remyelination – regenerative capacity of the adult brain

Demyelination, one of the hallmarks of the disease multiple sclerosis (MS), is the pathological process in which the myelin sheaths are lost or destroyed, leaving axons denuded. This type of demyelination is also commonly mentioned as primary demyelination, which should be distinguished from the term secondary demyelination or Wallerian degeneration, a process where myelin loss and degeneration occur as a consequence of primary axonal loss (Franklin and Goldman, 2015). Remarkably, adult CNS demyelination is often followed by a spontaneous regenerative process, namely remyelination, which is the restoration of myelin sheaths to demyelinated axons and the subsequent saltatory conduction is regained (Smith et al., 1979) leading to partial recovery of functional deficit (Duncan et al., 2009; Jeffery and Blakemore, 1997; Liebetanz and Merkler, 2006). Surprisingly, this process occurs with relatively high efficiency in animal models following experimental demyelination but also in humans with multiple sclerosis. The main reason for this notable endogenous repair capacity is thought to be due to the presence of the OPC population and their ability to proliferate and generate new oligodendrocytes and myelin. Animals that were locally irradiated prior to demyelination further suggested that remyelination required cell proliferation as they observed that remyelination was inhibited in these animals (Blakemore and Patterson, 1978). Nonetheless, the origin of the cells contributing to remyelination at the time remained inconclusive. Remyelination tissue has been observed to closely resemble normal physiologically myelinated tissue, with one exception – that the regenerated myelin sheaths are in general thinner and shorter than the initial myelin sheaths (Blakemore, 1974; Bunge et al., 1961). However, a more recent study has challenged this view, suggesting that after a much longer recovery time (6 months), newly remyelinated fibers have comparable myelin sheath length and thickness to axons myelinated developmentally (Powers et al., 2013). Interestingly, extensive remyelination has been observed to occur in some lesions in early stages of MS, but also to some extent at different stages of MS (Patrikios et al., 2006) Indeed, some of the neurological improvements seen during the remission may be due to this process. Although remyelination observed in MS can be quite extensive lesion-wise and geographically, its failure becomes progressively apparent as the disease develops (Patani et al., 2007; Patrikios et al., 2006).

Recent fate-mapping studies in experimental demyelinating models in rodents further support the view of OPCs generating new oligodendrocytes and myelin, underlying the remyelination process (Tripathi et al., 2010; Zawadzka et al., 2010). While contribution from existing mature oligodendrocytes appears not to be significant (Crang et al., 1998; Crawford et al., 2016; Keirstead and Blakemore, 1997). Moreover, a current study showed that the sustained activation of the extracellular signal-regulated kinase 1 and 2 (ERK1/2) in preexisting mature oligodendrocytes enables them to reinitiate myelination and contribute to the

remyelination process (Jeffries et al., 2016), showing that mature oligodendrocytes have the capacity of affecting myelin plasticity and repair. However, whether new oligodendrocytes also are involved in the demyelinating disease MS in humans in a similar fashion as in the rodents, or if existing mature oligodendrocytes are a potential contributor, it is currently not known and represent the focus of Paper II.

2.3 GRAY MATTER RENEWAL

2.3.1 Adult neurogenesis in humans

Contrary to the long-standing dogma that neurons cannot be generated in the adult brain, we now know that neurons are continuously generated in the adult mammalian brain. However, adult neurogenesis appears to occur only in discrete regions of the brain, namely in the olfactory bulb and the hippocampus (Frisén, 2016; Ming and Song, 2011). Most studies on the distribution and extent of adult neurogenesis have been performed in the adult rodent brain. It has been much harder to determine this in humans, as the traditional method employed is not appropriate for human use (see section 2.1), and consequently much less is known how adult neurogenesis occurs in the human brain.

Human adult neurogenesis has been investigated in several studies by quantifying cells with neuroblast (immature neuron) markers and studying morphology in human postmortem brain and in combination with the presence of any proliferating cells. A limited number of proliferating cells and a few migrating neuroblasts were identified in the human subventricular zone and rostral migratory stream (the path that ends in the olfactory bulb) (Sanai et al., 2011; Sanai et al., 2004; Wang et al., 2011). However, another study reported a much higher number of proliferating cells (Curtis et al., 2007). An intrinsic limitation of the neuroblast analysis in these studies is that the fate of these cells could not be concluded, that is if they become mature neurons and integrate long-term in the olfactory bulb. To overcome this limitation and elucidate the extent of adult olfactory bulb neurogenesis in humans we employed the ^{14}C-dating strategy to examine this as described in Paper III.

3 PRESENT INVESTIGATION

3.1 AIMS

The overall aim of this thesis is to investigate and elucidate the occurrence of cell renewal in the human brain.

The specific aims of the different studies are as follows:

Paper I

To assess whether there is any oligodendrocyte renewal in the human brain and to what extent this process occurs.

Paper II

To investigate how the generation dynamics of oligodendrocytes might be affected under pathological conditions such as in the demyelination disease multiple sclerosis.

Paper III

To examine the extent and the potential role of postnatal neurogenesis in the human olfactory bulb.

3.2 PAPER I

3.2.1 Summary of results

As described in section 2.2 of the thesis, myelination can be modified by mature oligodendrocytes generating new myelin and/or by exchanging oligodendrocytes and their myelin sheaths. Recent observations in animal studies support that continuous generation and exchange of oligodendrocytes contribute to myelin remodeling in the adult brain (Gibson et al., 2014; Young et al., 2013), though, it is less clear to what degree mature oligodendrocytes can modulate their myelination as well as to what extent this appears in the human brain. Therefore, in Paper I we addressed the question of how the dynamics of oligodendrocyte generation and myelination occurs in the human brain using our laboratory developed ^{14}C-dating strategy (Spalding et al., 2005).

Using quantitative stereology we found that the number of mature oligodendrocytes, identified by the combined expression of SOX10 (sex determining region Y- box 10) and NOGO-A (neurite outgrowth inhibitor-isoform A) in corpus callosum started to reach a plateau at ~5 years of age (88% of the final number) and after the age of 9, (98.5% of the final number of oligodendrocytes were reached). Although there was a slight but not significant trend of decrease with age, the oligodendrocyte numbers stayed largely constant throughout life. Further analysis of the myelin volume was performed in those individuals whose number of oligodendrocytes had been quantified. We discovered an initial steep increase in myelin volume during childhood, (at 5 years of age, 86% of the final volume was reached) which continued into adolescence, reaching its peak at the age of 17. A slow gradual decline followed during aging, similar to the observations of the total volume of the corpus callosum established by imaging (Lebel et al., 2012). Interestingly, after the age of 5, when the oligodendrocyte numbers started to plateau, we did not observe any correlation between the number of oligodendrocytes and the myelin volume. Thus, after the initial expansion phase, the major determinant of the myelin volume in humans does not appear to be the number of oligodendrocytes.

If there is a continuous generation of oligodendrocytes in human white matter as suggested from rodent studies (Young et al., 2013), a considerable part of human white matter cells would be newly generated and will be detectable by our carbon dating strategy. In order to explore this, we carbon-dated corpus callosum and frontal white matter cells. The ^{14}C concentration in the genomic DNA of the white matter cells corresponded to atmospheric ^{14}C levels a few years after birth in those individuals born after the atmospheric ^{14}C increase, indicating cell turnover, albeit little or limited. Measurements from individuals born before the onset of the increase, showed ^{14}C concentrations lower than at any time after 1955 (the nuclear bomb test increase), demonstrating that a substantial proportion of the cells in the white matter are very old and have not been replaced for decades. In contrast, when we

measured the levels of ^{14}C in biochemically-purified myelin, we found that in all measured individuals the ^{14}C levels in myelin corresponded to the atmospheric ^{14}C levels around the time of death of the individuals. This demonstrates that human myelin is contemporary and it is continually exchanged. This is consistent with studies in rodents revealing that myelin proteins are relatively stable and long-lived proteins (compared to nuclear pore complex proteins and histone proteins) (Savas et al., 2012; Toyama et al., 2013), although they are replaced within months.

Although a majority of the cells in white matter tracts are oligodendrocytes, it is significant to specifically isolate and birth date oligodendrocytes, since turnover dynamics likely may differ in diverse cell populations. Hence, we developed a strategy to specifically isolate oligodendrocyte nuclei by flow cytometry (fluorescence activated cell sorting, FACS). We isolated mature oligodendrocyte nuclei by the combined antibody labeling against SOX10, a general oligodendrocyte lineage marker (Stolt et al., 2002), and APC (adenomatous polyposis coli, monoclonal antibody CC1), a marker of mature myelinating oligodendrocytes (Bhat et al., 1996).

Further assessment by quantitative reverse transcription polymerase chain reaction (qRT-PCR) demonstrated high enrichment of three different mature myelinating oligodendrocyte markers and SOX10 in the SOX10+/APC+ fraction. Moreover, this fraction was almost devoid of markers for astrocytes, oligodendrocyte progenitors, microglia, endothelial cells and hematopoietic cells. Next, we measured the ^{14}C concentration in oligodendrocyte genomic DNA from corpus callosum. We found that in individuals born before the onset of the nuclear bomb test (1955), the concentration was higher than the pre-bomb atmospheric levels, showing that oligodendrocytes had been generated after the onset of the increase in ^{14}C. However, in 9 out of 10 individuals, the ^{14}C levels were lower than at any time after the increase, revealing that a considerable proportion of the oligodendrocyte must have been generated before the nuclear bomb tests (1955) and thus has existed for more than 50 years.

In contrast to our observation of little turnover of oligodendrocytes, extensive studies in mouse white matter have revealed a rather high generation of oligodendrocytes (Rivers et al., 2008; Young et al., 2013; Zhu et al., 2011). Hence, we performed additional analysis of the oligodendrocyte lineage cells by proliferation marker Ki-67 expression in human post mortem corpus callosum and thymidine analogue IdU in human post mortem gray and white matter tissue from frontal and occipital cortex. We detected very low numbers of proliferating oligodendrocyte progenitor cells (Ki-67+/SOX10+/NOGO-A-) after the perinatal period (age of 4) and rare generation of oligodendrocytes after one treatment of IdU administration (only 1 IdU-labeled mature oligodendrocyte in > 10 000 analyzed mature oligodendrocytes in each patient). This result confirmed the limited generation of oligodendrocytes after the early childhood period in humans in contrast to rodents.

With mathematical modeling ^{14}C data from individuals born before and after the nuclear bomb tests, we could provide an integrated view on cell turnover. Moreover, this enables the reproduction of the ^{14}C concentration a cell population would have provided it followed any certain pattern of cell generation and death. By testing different scenarios, we found that after an expansion phase (perinatally), the ^{14}C data was best explained by no substantial change in oligodendrocyte numbers after 5 years of age, providing an independent validation of the time course for the formation of the final number of oligodendrocytes in humans. Next, when computing which annual turnover rate would best fit all subjects together, we obtained the best fit with a model of constant turnover of 0.32% per year. Based on this model we could not find any correlation between turnover rate and age, which would motivate additional testing of different age dependent models. Oligodendrocytes could potentially be generated by direct differentiation of oligodendrocyte progenitor cells, without cell division (Hughes et al., 2013). We thus considered this possibility, but we found that this could increase the annual turnover rate maximally from 0.32% to 0.33%, due to the numbers of oligodendrocyte progenitor cells and their rather stable number in corpus callosum throughout life.

Regional differences in the oligodendrocyte population have recently been reported in the adult rodent brain (Dimou et al., 2008; Vigano et al., 2013). To examine if these differences may exist also in humans, we carbon dated genomic DNA from oligodendrocyte (SOX10+/APC+) nuclei isolated from prefrontal and frontal gray matter cortex. Interestingly, we found that subjects born before the onset of the atmospheric ^{14}C increase had higher concentration and individuals born after the increase had lower ^{14}C concentration in gray matter oligodendrocytes compared to white matter oligodendrocytes in the same subjects. This implies that indeed there might be a regional difference in the oligodendrocyte population as oligodendrocytes showed higher cell turnover in gray matter as compared to white matter oligodendrocytes. From the initial comparison of ^{14}C data between gray matter and white matter oligodendrocytes, it was already apparent that the gray matter oligodendrocyte population followed a different turnover dynamic. Mathematical modeling estimated that the gray matter oligodendrocytes have a more prolonged expansion phase in the cortex and expected it to continue until the fourth decade of life, with a subsequent annual turnover rate of 2.5%. Indeed, our observations showed that there is different oligodendrocyte turnover dynamics in white and gray matter.

In conclusion, our data provides evidence that human white matter oligodendrocytes are remarkably stable with limited turnover as compared to previous studies in mammals, whereas myelin is exchanged at a high rate. This implies that the limited turnover cannot underlie the white matter plasticity observed in humans in response to external stimuli. In contrast, myelin remodeling in humans may be carried out by mature oligodendrocytes. Our study opens up the possibility that myelination in white

matter is modulated in a conceptually different way in humans compared to the well-studied rodents. However, we also found a difference in oligodendrocyte generation and turnover kinetics in human gray matter, with a longer period of oligodendrocyte generation and higher turnover rate throughout life. This provides the possibility of de novo myelination in the sparsely myelinated cortex, suggesting that humans may utilize a different mechanism of myelination to modulate neural function and plasticity in different parts of the adult brain.

3.2.2 Discussion and future perspective

3.2.2.1 Humans – are we the same as mice?

The process of oligodendrocyte generation in rodents has been studied quite extensively, establishing a high degree of newborn oligodendrocytes and new myelin in the adult rodent white matter. Consistently with the observations in rodents, we found that human myelin is constantly renewed. However, our observation of 0.32% annual turnover of oligodendrocytes (after the age of five) in human white matter is at least 100-fold lower compared to adult mice (36.5%–182%/year, in the optic nerve and corpus callosum respectively, see Paper I for calculations). Although, oligodendrocyte generation in human gray matter showed a higher degree of turnover, gray matter oligodendrogenesis in rodents has not been as well studied, which makes any comparison between species difficult. An explanation of the differences observed could be due to the differences in the extent of myelination in white matter tracts. In white matter tracts not all axons are myelinated, but variability may occur in the number of myelinated axons between white matter tracts and within the same tract. In rodents the white matter tract, corpus callosum is largely unmyelinated, with around 30% of the axons being myelinated (Sturrock, 1980). A more recent study confirms this observation (Wang et al., 2008). In addition, they also reported that the degree of myelination (in corpus callosum) increases with brain size, further confirming that non-human primates have a much higher degree of myelination (~70–97%) than rodents (Lamantia and Rakic, 1990; Wang et al., 2008). Moreover, the human corpus callosum is shown to be almost fully myelinated (Aboitiz et al., 1992). However, the optic tract, an almost fully myelinated white matter region in rodents, still shows a dramatically higher turnover rate compared to humans, which suggests that human myelination may use a different mechanism to modulate myelin in white matter.

3.2.2.2 Dynamic myelination

In individuals practicing a new skill such as juggling (Scholz et al., 2009), after 6 weeks of intensive training, they showed a ~ 5% increase in FA (imaging parameter which correlates with myelin volume) in the engaged white matter region, reflecting that myelination is very dynamic and may be adapted by learning. Recently, the generation of new oligodendrocytes in the adult mouse brain has also been demonstrated to be a requirement in learning new tasks (McKenzie et al., 2014), proposing that myelination is modulated by new myelin from newly generated oligodendrocytes. With our low observed generation rate in humans, the exchange of oligodendrocytes cannot really account for the dynamic changes seen in white matter volume, in for example, learning and training paradigms. Moreover, our observations showed that after 5 years of age, there was no correlation between the number of oligodendrocytes and the myelin volume, nor is there any correlation between oligodendrocyte turnover rate and myelin volume in human white matter. Taken together, this implies that neither the oligodendrocyte numbers nor the replacement rate is a major determinant of myelin volume in human white matter. Instead, myelination may be regulated by modulating the thickness of the myelin sheets of mature oligodendrocytes in humans. It would be of great interest to further examine myelin sheath thickness and axon diameter during development, adulthood and aging in humans, as it could shed further light on how these parameters may modulate and optimize the function of neuronal circuitry.

3.2.2.3 Mechanisms of myelin plasticity

Have humans and rodents developed different mechanisms for myelin remodeling? In contrast to rodents, our data have established that in humans the generation of new myelin by new oligodendrocytes does not play a major role in myelin remodeling. While it has been shown that generation of oligodendrocytes contributes to myelin remodeling in rodents, it does not omit the possibility that mature oligodendrocytes in rodents are able to modulate their myelin. In mice where oligodendrocytes were genetically ablated of the phosphatase and tensin homolog (PTEN), it resulted in increased thickness of the myelin sheaths (Goebbels et al., 2010; Snaidero et al., 2014). Another recent study showed similar observation after a sustained activation of ERK1/2 in oligodendrocytes (Jeffries et al., 2016). This implicates that mature rodent oligodendrocytes have the capacity of modulating myelin sheath thickness. This could represent a more efficient way of modulating myelination, as the replacement of oligodendrocytes would inevitably result in a transitory demyelination of axons, which could likely impair function transiently.

3.2.2.4 Oligodendrocyte diversity?

Another growing question is oligodendrocyte lineage heterogeneity. Does this exist in humans? And in particular, is there heterogeneity among the myelinating oligodendrocytes in adult humans? Almost a century ago, Pio Del Rio-Hortega first observed and classified four types of oligodendrocytes based on morphology and their target axons (Iglesias-Rozas and Garrosa, 2012). More recently, region heterogeneity has been observed, with oligodendrocytes in mouse cerebral cortex making shorter internodes than their counterparts in other brain regions, such as corpus callosum and brain stem (Chong et al., 2012). Potential differences in electrical properties of oligodendrocytes have also been suggested (Bakiri et al., 2011). A transcriptional study in rodents by Marques and colleagues has further highlighted that such heterogeneity exists among oligodendrocytes in the adult CNS (Marques et al., 2016). The next challenge would be to investigate the heterogeneity in humans and how this diversity may influence myelination and the subsequent effect on neuronal function. Moreover, if such heterogeneity is evident, could the various oligodendrocytes be differently involved and affected in myelin diseases such as multiple sclerosis? The understanding of the heterogeneity of these cells will be crucial for a better knowledge of the pathology process in different myelin disorders and lead to the development of better target-effective therapies.

3.3 PAPER II

3.3.1 Summary of results

The hallmark of multiple sclerosis (MS) consists of loss of oligodendrocytes and myelin, as well as the axons, leading to conduction deficits and a variety of neurological symptoms. In early stages of MS, regeneration of myelin (remyelination) has been observed (Patrikios et al., 2006). In rodents it has been revealed that progenitor cells generate new myelinating oligodendrocytes contributing to the remyelination process (Tripathi et al., 2010; Zawadzka et al., 2010) and existing mature oligodendrocytes appear not to influence this process (Crawford et al., 2016). From our study described in Paper I, we found that adaptive myelin changes in humans may be carried out mainly by mature oligodendrocytes modulating their myelin, in contrast to rodents where it seems to be regulated mainly by oligodendrocyte exchange. Since there is a large difference observed in the dynamics of oligodendrocyte generation and myelination between rodents and humans (Paper I), how well does the different experimental animal models of MS reflect the human disease and cellular response to it? Thus, how this process is mediated in multiple sclerosis remains to be elucidated.

In Paper II, we investigate the cell generation dynamics in multiple sclerosis. To address this, we used our established ^{14}C-dating strategy (Spalding et al., 2005),

which takes the advantage of the nuclear bomb test generated-^{14}C in the atmosphere and assessed the age of mature oligodendrocytes in multiple sclerosis patients.

Using our previously established strategy of isolating mature oligodendrocyte nuclei from human postmortem tissue by FACS (Paper I), we successfully isolated and carbon dated mature oligodendrocyte nuclei from normal appearing white matter postmortem tissue of multiple sclerosis patients. When comparing the ^{14}C concentration in multiple sclerosis patients with healthy subjects born before the nuclear bomb test, there was no significant difference in the ^{14}C levels, revealing that the disease does not seem to affect the generation rate of oligodendrocytes. Interestingly, in some multiple sclerosis patients born after the atmospheric ^{14}C increase, mathematical modeling showed a highly elevated generation rate after disease onset compared to healthy individuals.

We further examined if this observation may be related to aging, since the ability of oligodendrocyte generation and remyelination decreased with age in animal models of multiple sclerosis (Shields et al., 1999; Sim et al., 2002). However, no correlation was observed between age at disease onset and the generation rate of oligodendrocytes in the multiple sclerosis patients.

Moreover, with our ^{14}C dating technique we would be able to detect if older multiple sclerosis patients born before the atmospheric ^{14}C increase would have had an increased oligodendrocyte generation rate the first decade after their disease onset. As the early disease period overlaps with the highly elevated atmospheric ^{14}C levels, it would be readily detectable even after a long time due to the cumulative property of ^{14}C. However, this was not observed in our patients. Instead, we found an inverse correlation between the time from disease onset to the patient's death and the oligodendrocyte generation rate in the multiple sclerosis patients. The MS patients with an increased oligodendrocyte generation rate had a common factor of a rapid progression to death and likely a very aggressive disease. Thus, the patients with the most aggressive disease died at a young age and it is probably why this effect was detected in the latest born individuals. While all MS patients with an increased oligodendrocyte generation rate had a rapid disease progression, we also observed other patients with a similar rapid disease progression without exhibiting an elevated level of oligodendrocyte generation, reflecting that multiple sclerosis or the response to the disease and its progress is quite heterogeneous.

We next assessed the age of mature oligodendrocytes from remyelination lesions in white matter of MS patients. If oligodendrocyte generation contributes to remyelination as has been observed in rodents, this would suggest an increased oligodendrocyte generation during the disease period in remyelination lesion in MS patients born before the nuclear bomb tests and subsequently yield an elevated ^{14}C value. Remarkably, we found that the ^{14}C levels in oligodendrocyte nuclei from

remyelination lesions in patients born before the rapid increase of atmospheric ^{14}C concentration were not higher, but in contrast lower, than white matter from healthy subjects and normal appearing white matter of MS patients. This suggests that during the disease period the generation of new oligodendrocytes has been reduced and the existing oligodendrocytes were mainly generated before the onset of the disease. To test this possibility, we modeled a scenario to see how the absence of oligodendrocyte generation would affect the ^{14}C concentration after the disease onset. The modeling calculation closely resembled the measured ^{14}C concentration in mature oligodendrocytes from remyelination lesion, supporting the concept of inefficient oligodendrocyte generation in MS white matter lesion.

We further examined if oligodendrocyte progenitor cells could have directly differentiated to oligodendrocytes without proliferation (Hughes et al., 2013), as this would lead to an underestimation of oligodendrocyte generation and give the impression that the oligodendrocytes are old even though they would be newly differentiated. However, we found that oligodendrocyte progenitor cells in normal appearing white matter were younger than the mature oligodendrocytes in remyelination lesion. Subsequently, if the progenitor would differentiate directly to mature oligodendrocytes, the oligodendrocytes would still appear significantly younger than what we observed. Moreover, the number of oligodendrocytes progenitor cells is around 20-fold less than the mature oligodendrocytes in adult white matter (Paper I) and to be able to reconstitute the amount of mature oligodendrocytes it would require more than four cell divisions of the progenitor cells. We tested this scenario with mathematical modeling and found that even with very conservative estimates, the calculated values would be detected as substantially different from the measured ^{14}C concentration in mature oligodendrocytes from remyelination lesion. Therefore, we could exclude this possibility.

In summary, we found that in normal appearing white matter the oligodendrocyte dynamics seem not to be affected in MS, except for a subset of patients with an aggressive disease where an increased oligodendrocyte generation was observed. Our data demonstrate that human white matter has an intrinsic potential to largely increase oligodendrocyte generation, but it fails with increasing durations of the disease and in MS lesions. Furthermore, we revealed that oligodendrocyte generation is mainly absent in remyelinated lesions, establishing that remyelination in MS patients is carried out by old spared oligodendrocytes.

3.3.2 Discussion and future perspective

3.3.2.1 Newly formed oligodendrocytes contributing to remyelination?

The CC-1 (anti-APC, monoclonal clone, CC1) antibody was recently revealed not to bind APC, but found to bind Quaking-7 (QKI-7), a RNA-binding protein, highly

expressed in mature myelinating oligodendrocytes. However, in the study by Marques and colleagues, QKI-7 was also expressed in relatively high level in the population defined as pre-myelinating oligodendrocytes (NFOL1 and NFOL2) in their single cell mouse transcription expression analysis data set (Marques et al., 2016) (http://linnarssonlab.org/oligodendrocytes/). Moreover, there was a preliminary observation of a fraction of the pre-myelinating oligodendrocytes (ITPR2+ cells) being labeled with the CC-1 antibody in the adult mouse brain (S. Marques, personal communication, 2017). They also observed an increase in number of this population when learning a new skill (learn to run in a complex wheel) in line with a previous mouse study (McKenzie et al., 2014), which showed that the early response in learning a skill involved newly formed oligodendrocytes. One could therefore speculate on the possibility of these newly formed, pre-myelinating oligodendrocytes contributing to remyelination as an early response in MS.

Thus, we cannot exclude that our sorted SOX10+/CC-1+ fraction, that we carbon dated may include pre-myelinating oligodendrocytes. However, our ^{14}C data from remyelination lesions showed that the SOX10+/CC1+ oligodendrocytes were very old, simply confirming that if there are pre-myelinating oligodendrocytes contributing to the remyelination, they are indeed very old, being generated postnatally and having survived for at least 3-4 decades. Furthermore, in mouse white matter the ITPR2+ pre-myelinating oligodendrocyte population constitutes around ~15% of the oligodendrocyte lineage cells (SOX10+): does this population exist in humans? If so, how large is this population in the human brain? Is there a similar regional diversity as observed in the mouse? In fact, there is currently no established marker for this population in humans and additional characterization of these pre-myelinating oligodendrocytes in humans is needed before we can further investigate the potential role of this population in the remyelination process in multiple sclerosis patients.

3.3.2.2 Remyelination by existing oligodendrocytes

In MS, myelin sheaths are being attacked by the immune system (Ransohoff et al., 2015). The autoimmune reaction in MS can be directed against a wide variety of epitopes in different MS patients and several of the autoantigens are epitopes mainly associated with the myelin sheaths, while others are related to the cell body of oligodendrocytes or expressed by non-oligodendrocytes (Fraussen et al., 2014). In early MS lesions, Romanelli and colleagues observed a loss of myelin, which would also indicate a loss of oligodendrocytes (Romanelli et al., 2016). However, quantification analysis revealed that the number of mature oligodendrocytes remained unchanged compared to normal appearing white matter (Romanelli et al., 2016). This implied a selective destruction of the myelin sheaths by myelin autoantibodies and, as long as the oligodendrocyte cell bodies were left intact, the oligodendrocytes can potentially regenerate their myelin sheaths and remyelination

may occur. Moreover, a remyelination study in rodents observed that newly remyelinated fibers produced by new oligodendrocytes can achieve myelin sheath length and thickness similar to developmentally myelinated axons (Powers et al., 2013). Their observations suggested that the long-lasting abnormally thin myelin observed in pathological lesions, such as in MS remyelination lesions, unlikely is the result of incomplete myelin remyelination (from newly generated oligodendrocytes). Instead, it may be due to myelin re-modulation from preexisting oligodendrocytes (Powers et al., 2013). Together, these suggestions are in line with our findings that spared oligodendrocytes can contribute to remyelination in MS.

It should be noted that our findings are in contrast with the current view on the remyelination process, which is mainly inferred from rodent studies, where remyelination is primarily being carried out by the generation of new oligodendrocytes. Our findings highlight that humans may use a different mechanism in myelin repair than what have been observed in animal studies. Furthermore, our data suggest that immediate therapies inhibiting the destruction of oligodendrocytes and identification of molecular regulator affecting myelin of existing oligodendrocyte may be crucial for remyelination and subsequently functional recovery.

3.4 PAPER III

3.4.1 Summary of results

Olfactory bulb neurogenesis has been studied extensively in rodents, with large numbers of neuroblasts migrating every day from the subventricular zone to the olfactory bulb (Ming and Song, 2011) and approximately 40% of the newly generated neurons integrate into the olfactory bulb circuitry and survive long-term (Winner et al., 2011). This process appears not to be restricted to rodents, but has been shown to occur also in adult non-human primates (Kornack and Rakic, 2001) . However, the extent and potential role of postnatal olfactory bulb neurogenesis in humans have been questioned, as there have been contradicting results from recent human studies (Curtis et al., 2007; Sanai et al., 2011; Sanai et al., 2004). In Paper III, we set out to investigate the occurrence of adult neurogenesis in the olfactory bulb by ^{14}C birth dating.

To understand if there is any postnatal generation in the olfactory bulb we first carbon-dated adult human olfactory bulb cells. The ^{14}C levels in genomic DNA of human postmortem olfactory bulb from all individuals indicated significant cell turnover in the human olfactory bulb. However, the human olfactory bulb consists of almost equal numbers of neurons and non-neuronal cells, and therefore it is not possible to discriminate if the observed cell turnover occurs in all cells or if it may be restricted to one of these populations.

To specifically determine the age and turnover of neurons and non-neuronal cells, we used the antibody against NeuN (neuronal nuclei) to isolate neuronal nuclei by FACS. Even if the neuronal marker NeuN is a well-established marker for most neuronal subtypes, it does not label the mitral and certain glomerular layer neurons in the rodent olfactory bulb (Mullen et al., 1992). Moreover, in histological analysis of human olfactory bulb, we also found that a subset of neurons were NeuN-. In order not to exclude any neuronal subtypes, we found that HuD antibody specifically labeled all post-mitotic neurons, thus allowing the isolation of neuronal nuclei from the human olfactory bulb. However, histological analysis revealed that in addition to neurons, the HuD antibody also labels a subset of non-neuronal cells. The non-neuronal cells labeled by the HuD antibody had oligodendrocyte lineage characteristics and they were further confirmed to co-express SOX10 and CNPase (2',3'-cyclic nucleotide 3'-phosphodiesterase). We subsequently isolated neuronal nuclei (HuD+/SOX10-) and non-neuronal nuclei (HuD- and or NeuN-) by flow cytometry and measured the ^{14}C concentration in genomic DNA in these populations.

The ^{14}C data together with mathematical modeling revealed that the non-neuronal population has an annual turnover rate of 2.0%-3.4%. Since this represents an average for all non-neuronal cells, it is likely that the turnover dynamics may vary between specific non-neuronal cell types. This was also observed when modeling the data. The global model fit for the non-neuronal cells was not optimal and indicated that turnover rates may change with aging or that the population is heterogeneous, with cells having different turnover rates. One may speculate if the HuD-/SOX10+ oligodendrocytes potentially could have a higher turnover rate than what has been observed in other regions of human white and gray matter (0.3% and 2.5%, respectively). However, in this study the HuD-/SOX10+ population was not separated from the other HuD/SOX10- non-neuronal cells. Moreover SOX10+ is a general oligodendrocyte lineage marker and it may also include oligodendrocyte progenitor cells as shown in Paper I. A more plausible explanation to the considerable turnover in the non-neuronal populations could come from microglia turnover, where it has been described to have an annual turnover rate of 28% in human cortex (Reu et al., unpublished data).

In contrast, we found that the genomic DNA ^{14}C concentrations of neurons corresponded to atmospheric levels at the time around the birth of the individual, indicating either the absence or an extremely low level of postnatal olfactory bulb neurogenesis. We could not exclude a limited constant turnover of neurons with an annual turnover rate of 0.008%. This would be as if less than 1% of all neurons would be replaced after a lifetime of 100 years.

In sum, we report that human olfactory bulb neurons are almost as old as the individuals, establishing that there is very limited, if any, postnatal neurogenesis in

the human olfactory bulb. This highlights a fundamental difference in the plasticity of the human brain compared to other mammals and it reminds us that we need to be cautious whether studies in rodents could be correctly generalized to the human brain function and disease.

3.4.2 Discussion and future perspective

Psychiatric and addiction disorders have been suggested to decrease olfactory bulb neurogenesis (Hansson et al., 2010; Negoias et al., 2010; Winner et al., 2011). In our data set some of the individuals were diagnosed with some of these conditions (Paper III). However, in all individuals observed, the ^{14}C concentration in olfactory bulb neurons corresponded to the time around birth and no apparent correlation were found between olfactory bulb neurogenesis and these conditions. Moreover, in several neurodegenerative diseases, a common and early symptom is anosmia, which has been implied to be associated with reduced adult olfactory bulb neurogenesis (Höglinger et al., 2004; Winner et al., 2011), but our findings show that this seems unlikely.

In rodents it has been estimated that around 50% of the olfactory bulb neurons are replaced annually (Imayoshi et al., 2008) and if we would compare our finding with rodents, the extent of human postnatal olfactory bulb neurogenesis is order of magnitudes lower. This difference may probably reflect a functional brain difference, as from rodent studies it has been suggested that adult born neurons contribute to odorant discrimination and olfactory learning and memory formation (Lazarini and Lledo, 2011; Magavi et al., 2005). However, the lack of equivalent adult generation of olfactory bulb neurons in humans could suggest that these functions may be differently mediated in humans or just the simple fact that maybe humans are not as olfaction-dependent as the rodents.

4 CONCLUDING REMARKS

In this thesis I have presented how renewal of cells in the brain may affect brain plasticity and in particular white matter plasticity.

Furthermore this thesis has shed light on the importance of human studies, as our results have revealed large differences in neuronal cell and oligodendrocyte generation between humans and rodents. Although we cannot undermine the significance of animal studies, as it is essential in advancing our understanding of the function of oligodendrocytes, myelin and neurons in the adult brain, it is however, crucial to consider species differences. These differences are especially relevant when the role of cell turnover in diseases is investigated, as animals and in particular rodent models may not always translate the human situation well.

As illustrated by the works presented in this thesis I hope I have made it apparent that oligodendrocytes play a larger role than only just being a support cell and a static insulator of axons. Indeed they have an emerging role in modulating neural plasticity and the complex function of the brain. Rodent studies have suggested that adaptive myelin modulation is mainly executed by the generation of new oligodendrocytes. However, our findings have challenged this prevailing view. Our results have highlighted the possibility and role of existing mature oligodendrocytes in modulating their myelin and identifying another mechanism of adaptive modulation of myelin that may occur in the human brain in contrast to what has been observed in rodents. Moreover this further emphasizes that myelin is starting to become considered a highly dynamic and active element of the central nervous system, with an increasing role as a key player in neural circuitry modulation and in adult myelin plasticity.

5 ACKNOWLEDGEMENTS

My road to graduation has at times been bumpy and challenging, but it has always been a great treat because of all the inspiring, dedicated and talented people that have made this possible. It has been a privilege and I would like to express my deepest gratitude to all the people that directly, or indirectly contributed to this work and for all the support during these years.

Jag vill först och främst tacka min handledare **Jonas** för allt under de här åren. Det var varit otroligt lärorikt och tack för att jag ha fått kunna utföra fantastisk forskning i ett toppenlabb! Din dörr har alltid varit öppen för frågor och diskussioner och jag är djupt tacksam för allt stöd under alla år. För din otröttliga passion och entusiasm för forskning och vetenskap och ditt oändliga tålamod. Tack!

Kirsty, my co-supervisor, thanks for showing me and sharing the secrets of carbon dating and all the corridor-chats about everything else. Special thanks for proofreading my thesis.

Marie, min bihandledare, evigt tacksam för att du entusiastiskt visade den ovetande gymnasiestudenten stamcellers kapacitet och för att du introducerade mig till labbet och forskningsvärlden samt att du alltid funnits här och stöttat mig genom åren och varit tillgänglig för att prata om allt.

My collaborators at Uppsala University, **Göran Possnert** and **Mehran,** tack för all AMS- och kol-relaterad hjälp! Det lättar alltid upp att få dina glad mail-hälsningar!

My collaborators, **Lou**, tack för all hjälp och stöd under de här åren och alla frågor kring MS! **Cynthia**, thank you for your positivity, for all the short but great times, for hanging out at conferences and of course for all the struggle with the EAE mice. **Rux**, thanks for all the help with EAE and being such a great collaborator!

Past and present members of the **Frisén lab**, thanks for making the lab such a great place to work, day and night! **Jens**, for your optimism, the de-stressing reminders and discussion within and beyond science topics. Good luck with you thesis writing! **Pedro**, for all the helpful PhD application tips, for being such a happy person and for your encouraging laughter and bad jokes. ☺ **Johanna**, for all the conversations and being such a sweet person, **Moa,** for sharing similar passion for coffee and great company during the night shift at lab, **Joanna**, for you positive spirit and your corridor echoing laughter, **Embla**, for bringing the Frisén bar to the lab and all the modeling, **Giuseppe** and **Margeritha** for bringing great spirit. Extra big thanks to **Marta** for proofreading in such a short time! Grazie Mille! **Olaf**, for discussions and sharing ^{14}C secrets, **Jeff** for all the discussions and making the lab American, **Mehdi**, for all the help during my maternity leave, thanks! Thank you **James** for all

the help with the microscopes and the mice related discussions, **Kanar**, for all the help with donation cases and general chats, **Sofia**, for all the help with mathematical modeling and for sharing my passion for stockpiling sweets at lab, **Hanna** for all the laughter and support, **Dinos**, for your help and introduction to animal experiments, **Bella**, for being my first student. Thanks for all your hard work! Also thanks to **Enric**, **Michael**, **Patrik**, **Hagen**, **Aleksandra**, **Tadashi**, **Aurélie**, **Maria**, **Malin**, **Sarah**, **Anna** and all the past and present students!

Muchas gracias **Marcelo** and ευχαριστώ πολύ **Sarantis**, my FACS heroes, for making the tedious FACS sorting fun and effortless. ☺ **Helena,** for fixing and keeping the lab in order, taking care of us, the mice, my plumeria and for creating such a great atmosphere even on a dull Monday morning. ☺ Tusen tack!

Thanks to **Andras** and everyone in the **Simon lab**!

Thanks to **Christian** and everyone in the **Göritz lab**, for great chats, lunch and fika times! **David,** my previous office mate. Thanks for everything throughout the years! ☺ **Bettina**, for your curly hair and smiley face! **Sanyia**, for being such a sweetheart. Also thanks to **Daniel**, **Yildiz** and **Eduardo**.

Thanks to **Qian** and **Fu** in the **Spalding lab**!

All the past and present people at CMB and Ludwig institute, **Belma**, **Minerva**, **Karin**, **Vilma**, **Nigel**, **Stuart**, **Daniel**, **Cecile**, **Ingrid** and many more. Thanks for all the lunch, fika and good times!

Thank you **Matti**, for all the fast and skillful help throughout the years! **Christine**, för alla ekonomirelaterade frågor och roliga pratstunder, **Zdravko** and **Irene** for all help with logistics.

Tack **Mattias Karlén** for your quick help and awesome cover!

My friends outside science, **Norra-knattarna+1**, thanks for all the fun we have been up to since high school, and for reminding me that there is a world outside science too. A special thanks to **Sofia H** for being such a good listener and great friend.

Thanks to **my family** and especially my godparents, **契爺**, **契媽**, for all the help and support and for making this thesis possible. **契爺**, **契媽**, 多謝你們抽出寶貴時間照顧和幫助。 <3

Caroline, for helping me with Gabriel and taking your time proofreading my thesis. Tusen tack!

Mami, I cannot thank you enough for your never-ending help and support and for always being there for me! **媽咪**, 多謝你無限和永久的支持!!! <3

Gabu-chan, my cutiepie, my everyday sunshine and energizer. Mammas sötaste lilla böna och min alltid glada lilla solstråle! <3

And last but not least, my **Jå-chan** for always being with me i vått och torrt! Vi kan klara allt tillsammans! Fighting!! よろしくおねがいします, 末永く。<3

6 REFERENCES

Aboitiz, F., Scheibel, A.B., Fisher, R.S., and Zaidel, E. (1992). Fiber composition of the human corpus callosum. Brain Research *598*, 143-153.

Altman, J. (1962). Are New Neurons Formed in the Brains of Adult Mammals? Science *135*, 1127-1128.

Bakiri, Y., Káradóttir, R., Cossell, L., and Attwell, D. (2011). Morphological and electrical properties of oligodendrocytes in the white matter of the corpus callosum and cerebellum. The Journal of Physiology *589*, 559-573.

Barrera, K., Chu, P., Abramowitz, J., Steger, R., Ramos, R.L., and Brumberg, J.C. (2013). Organization of myelin in the mouse somatosensory barrel cortex and the effects of sensory deprivation. Developmental Neurobiology *73*, 297-314.

Bengtsson, S.L., Nagy, Z., Skare, S., Forsman, L., Forssberg, H., and Ullen, F. (2005). Extensive piano practicing has regionally specific effects on white matter development. Nature Neuroscience *8*, 1148-1150.

Bergmann, O., Bhardwaj, R.D., Bernard, S., Zdunek, S., Barnabe-Heider, F., Walsh, S., Zupicich, J., Alkass, K., Buchholz, B.A., Druid, H.*, et al.* (2009). Evidence for cardiomyocyte renewal in humans. Science *324*, 98-102.

Bergmann, O., Liebl, J., Bernard, S., Alkass, K., Yeung, M.S., Steier, P., Kutschera, W., Johnson, L., Landen, M., Druid, H.*, et al.* (2012). The age of olfactory bulb neurons in humans. Neuron *74*, 634-639.

Bhardwaj, R.D., Curtis, M.A., Spalding, K.L., Buchholz, B.A., Fink, D., Björk-Eriksson, T., Nordborg, C., Gage, F.H., Druid, H., Eriksson, P.S.*, et al.* (2006). Neocortical neurogenesis in humans is restricted to development. Proceedings of the National Academy of Sciences *103*, 12564-12568.

Bhat, R.V., Axt, K.J., Fosnaugh, J.S., Smith, K.J., Johnson, K.A., Hill, D.E., Kinzler, K.W., and Baraban, J.M. (1996). Expression of the APC tumor suppressor protein in oligodendroglia. Glia *17*, 169-174.

Blakemore, W.F. (1974). Pattern of remyelination in the CNS. Nature *249*, 577-578.

Blakemore, W.F., and Patterson, R.C. (1978). Suppression of remyelination in the CNS by X-irradiation. Acta Neuropathologica *42*, 105-113.

Blumenfeld-Katzir, T., Pasternak, O., Dagan, M., and Assaf, Y. (2011). Diffusion MRI of structural brain plasticity induced by a learning and memory task. PloS One *6*, e20678.

Boullerne, A. (2016). The history of myelin. Experimental Neurology *283*, 431-445.

Brody, B., Kinney, H.C., Kloman, A.S., and Gilles, F.H. (1987). Sequence of Central Nervous System Myelination in Human Infancy. I. An Autopsy Study of Myelination. Journal of Neuropathology and Experimental Neurology *46*, 283.

Bunge, M.B., Bunge, R.P., and Pappas, G.D. (1962). Electron microscopic demonstration of connections between glia and myelin sheaths in the developing mammalian central nervous system. The Journal of Cell Biology *12*, 448.

Bunge, M.B., Bunge, R.P., and Ris, H. (1961). Ultrastructural study of remyelination in an experimental lesion in adult cat spinal cord. The Journal of Biophysical and Biochemical cytology *10*, 67-94.

Chang, A., Nishiyama, A., and Peterson, J. (2000). NG2-positive oligodendrocyte progenitor cells in adult human brain and multiple sclerosis lesions. The Journal of Neuroscience *20*, 6404-6412

Chong, C.S.Y., Rosenberg, S.S., Fancy, S.P.J., Zhao, C., Shen, Y.-A.A., Hahn, A.T., McGee, A.W., Xu, X., Zheng, B., Zhang, L.I.*, et al.* (2012). Neurite outgrowth inhibitor Nogo-A establishes spatial segregation and extent of oligodendrocyte myelination. Proceedings of the National Academy of Sciences *109*, 1299-1304.

Crang, A.J., Gilson, J., and Blakemore, W.F. (1998). The demonstration by transplantation of the very restricted remyelinating potential of post-mitotic oligodendrocytes. Journal of Neurocytology *27*, 541-553.

Crawford, A.H., Tripathi, R.B., Foerster, S., McKenzie, I., Kougioumtzidou, E., Grist, M., Richardson, W.D., and Franklin, R.J.M. (2016). Pre-Existing Mature Oligodendrocytes Do Not Contribute to Remyelination following Toxin-Induced Spinal Cord Demyelination. The American Journal of Pathology *186*, 511-516.

Curtis, M.A., Kam, M., Nannmark, U., Anderson, M.F., Axell, M.Z., Wikkelso, C., Holtas, S., van Roon-Mom, W.M., Bjork-Eriksson, T., Nordborg, C.*, et al.* (2007). Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. Science *315*, 1243-1249.

Dawson, M., Polito, A., Levine, J.M., and Reynolds, R. (2003). NG2-expressing glial progenitor cells: an abundant and widespread population of cycling cells in the adult rat CNS. Molecular and Cellular Neuroscience *24*, 476-488.

Deoni, S.C.L., Mercure, E., Blasi, A., Gasston, D., Thomson, A., Johnson, M., Williams, S.C.R., and Murphy, D.G.M. (2011). Mapping Infant Brain Myelination with Magnetic Resonance Imaging. The Journal of Neuroscience *31*, 784-791.

Dimou, L., Simon, C., Kirchhoff, F., Takebayashi, H., and Gotz, M. (2008). Progeny of Olig2-expressing progenitors in the gray and white matter of the adult mouse cerebral cortex. The Journal of Neuroscience *28*, 10434-10442.

Duncan, I.D., Brower, A., Kondo, Y., Curlee, J.F., and Schultz, R.D. (2009). Extensive remyelination of the CNS leads to functional recovery. Proceedings of the National Academy of Sciences *106*, 6832-6836.

Eriksson, P.S., Perfilieva, E., Björk-Eriksson, T., Alborn, A.-M., Nordborg, C., Peterson, D.A., and Gage, F.H. (1998). Neurogenesis in the adult human hippocampus. Nature Medicine *4*, 1313-1317.

Ernst, A., Alkass, K., Bernard, S., Salehpour, M., Perl, S., Tisdale, J., Possnert, G., Druid, H., and Frisén, J. (2014). Neurogenesis in the Striatum of the Adult Human Brain. Cell *156*, 1072-1083.

Fields, R.D. (2008). White matter in learning, cognition and psychiatric disorders. Trends in neurosciences *31*, 361-370.

Fields, R.D. (2010). Neuroscience. Change in the brain's white matter. Science (New York, NY) *330*, 768-769.

Franklin, R.J., and Goldman, S.A. (2015). Glia Disease and Repair-Remyelination. Cold Spring Harbor perspectives in biology *7*, a020594.

Fraussen, J., Claes, N., de Bock, L., and Somers, V. (2014). Targets of the humoral autoimmune response in multiple sclerosis. Autoimmunity Reviews *13*, 1126-1137.

Frisén, J. (2016). Neurogenesis and Gliogenesis in Nervous System Plasticity and Repair. Annual review of cell and developmental biology *32*, 127-141.

Geren, B.B. (1954). The formation from the schwann cell surfaceof myelin in the peripheral nerves of chick embryos. Experimental cell research *7*, 558-562.

Gibson, E.M., Purger, D., Mount, C.W., Goldstein, A.K., Lin, G.L., Wood, L.S., Inema, I., Miller, S.E., Bieri, G., Zuchero, J.B.*, et al.* (2014). Neuronal Activity Promotes Oligodendrogenesis and Adaptive Myelination in the Mammalian Brain. Science *344*, 1252304.

Glasser, M.F., and Essen, V.D.C. (2011). Mapping human cortical areas in vivo based on myelin content as revealed by T1-and T2-weighted MRI. The Journal of Neuroscience 31, 11597-11616.

Goebbels, S., Oltrogge, J.H., Kemper, R., Heilmann, I., Bormuth, I., Wolfer, S., Wichert, S.P., Mobius, W., Liu, X., Lappe-Siefke, C.*, et al.* (2010). Elevated phosphatidylinositol 3,4,5-trisphosphate in glia triggers cell-autonomous membrane wrapping and myelination. The Journal of Neuroscience *30*, 8953-8964.

Gratzner, H.G. (1982). Monoclonal antibody to 5-bromo- and 5-iododeoxyuridine: A new reagent for detection of DNA replication. Science *218*, 474-475.

Hans, F., and Dimitrov, S. (2001). Histone H3 phosphorylation and cell division. Oncogene *20,* 3021-3027.

Hansson, A.C., Nixon, K., Rimondini, R., Damadzic, R., Sommer, W.H., Eskay, R., Crews, F.T., and Heilig, M. (2010). Long-term suppression of forebrain neurogenesis and loss of neuronal progenitor cells following prolonged alcohol dependence in rats. Int J Neuropsychopharmacol *13*, 583-593.

Hartline, D.K., and Colman, D.R. (2007). Rapid conduction and the evolution of giant axons and myelinated fibers. Current Biology *17*, 35.

Hildebrand, C., Remahl, S., Persson, H., and Bjartmar, C. (1993). Myelinated nerve fibres in the CNS. Progress in Neurobiology *40*, 319-384.

Höglinger, G.U., Rizk, P., Muriel, M.P., Duyckaerts, C., Oertel, W.H., Caille, I., and Hirsch, E.C. (2004). Dopamine depletion impairs precursor cell proliferation in Parkinson disease. Nature Neuroscience *7*, 726-735.

Hughes, E.G., Kang, S.H., Fukaya, M., and Bergles, D.E. (2013). Oligodendrocyte progenitors balance growth with self-repulsion to achieve homeostasis in the adult brain. Nature Neuroscience *16*, 668-676.

Huttner, H.B., Bergmann, O., Salehpour, M., Rácz, A., Tatarishvili, J., Lindgren, E., Csonka, T., Csiba, L., Hortobágyi, T., Méhes, G.*, et al.* (2014). The age and genomic integrity of neurons after cortical stroke in humans. Nature Neuroscience *17*, 801-803.

Huxley, A.F., and Stämpeli, R. (1949). Evidence for saltatory conduction in peripheral myelinated nerve fibres. The Journal of Physiology 108, 315-339.

Iglesias-Rozas, J.R., and Garrosa, M. (2012). The discovery of oligodendroglia cells by Rio-Hortega: his original articles. Clinical Neuropathology *31*, 437-439.

Imayoshi, I., Sakamoto, M., Ohtsuka, T., Takao, K., Miyakawa, T., Yamaguchi, M., Mori, K., Ikeda, T., Itohara, S., and Kageyama, R. (2008). Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain. Nature Neuroscience *11*, 1153-1161.

Jeffery, N.D., and Blakemore, W.F. (1997). Locomotor deficits induced by experimental spinal cord demyelination are abolished by spontaneous remyelination. Brain *120*, 27-37.

Jeffries, M.A., Urbanek, K., Torres, L., Wendell, S.G., Rubio, M.E., and Fyffe-Maricich, S.L. (2016). ERK1/2 Activation in Preexisting Oligodendrocytes of Adult Mice Drives New Myelin Synthesis and Enhanced CNS Function. The Journal of Neuroscience : the official journal of the Society for Neuroscience *36*, 9186-9200.

Kang, S.H., Fukaya, M., Yang, J.K., Rothstein, J.D., and Bergles, D.E. (2010). NG2+ CNS glial progenitors remain committed to the oligodendrocyte lineage in postnatal life and following neurodegeneration. Neuron *68*, 668-681.

Keirstead, H.S., and Blakemore, W.F. (1997). Identification of post-mitotic oligodendrocytes incapable of remyelination within the demyelinated adult spinal cord. J Neuropathol Exp Neurol *56*, 1191-1201.

Keller, T.A., and Just, M. (2009). Altering cortical connectivity: remediation-induced changes in the white matter of poor readers. Neuron *64*, 624-631.

Kessaris, N., Fogarty, M., Iannarelli, P., Grist, M., Wegner, M., and Richardson, W.D. (2006). Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage. Nature Neuroscience *9*, 173-179.

Kornack, D.R., and Rakic, P. (2001). The generation, migration, and differentiation of olfactory neurons in the adult primate brain. Proceedings of the National Academy of Sciences *98*, 4752-4757.

Lamantia, A.S., and Rakic, P. (1990). Cytological and quantitative characteristics of four cerebral commissures in the rhesus monkey. The Journal of comparative neurology *291*, 520-537.

Lazarini, F., and Lledo, P.M. (2011). Is adult neurogenesis essential for olfaction? Trends Neurosci *34*, 20-30.

Lebel, C., Gee, M., Camicioli, R., Wieler, M., and Martin, W. (2012). Diffusion tensor imaging of white matter tract evolution over the lifespan. Neuroimage 60, 340-352.

Levin, I., and Kromer, B. (2004). The tropospheric 14CO2 level in mid latitudes of the northern hemisphere (1959-2003). Radiocarbon *46*, 1261-1272.

Liebetanz, D., and Merkler, D. (2006). Effects of commissural de- and remyelination on motor skill behaviour in the cuprizone mouse model of multiple sclerosis. Experimental neurology *202*, 217-224.

Liu, J., Dietz, K., DeLoyht, J.M., Pedre, X., Kelkar, D., Kaur, J., Vialou, V., Lobo, M.K., Dietz, D.M., Nestler, E.J.*, et al.* (2012). Impaired adult myelination in the prefrontal cortex of socially isolated mice. Nature Neuroscience *15*, 1621-1623.

Magavi, S.S.P., Mitchell, B.D., Szentirmai, O., Carter, B.S., and Macklis, J.D. (2005). Adult-Born and Preexisting Olfactory Granule Neurons Undergo Distinct Experience-Dependent Modifications of their Olfactory Responses In Vivo. The Journal of Neuroscience *25*, 10729-10739.

Makinodan, M., Rosen, K.M., Ito, S., and Corfas, G. (2012). A critical period for social experience-dependent oligodendrocyte maturation and myelination. Science *337*, 1357-1360.

Marques, S., Zeisel, A., Codeluppi, S., van Bruggen, D., Mendanha Falcão, A., Xiao, L., Li, H., Häring, M., Hochgerner, H., Romanov, R.A.*, et al.* (2016). Oligodendrocyte heterogeneity in the mouse juvenile and adult central nervous system. Science *352*, 1326-1329.

McKenzie, I.A., Ohayon, D., Li, H., Paes de Faria, J., Emery, B., Tohyama, K., and Richardson, W.D. (2014). Motor skill learning requires active central myelination. Science *346*, 318-322.

Mehta, M.A., Golembo, N.I., Nosarti, C., Colvert, E., Mota, A., Williams, S.C.R., Rutter, M., and Sonuga-Barke, E.J.S. (2009). Amygdala, hippocampal and corpus callosum size following severe early institutional deprivation: The English and Romanian Adoptees Study Pilot. Journal of Child Psychology and Psychiatry *50*, 943-951.

Miller, D.J., Duka, T., Stimpson, C.D., Schapiro, S.J., Baze, W.B., McArthur, M.J., Fobbs, A.J., Sousa, A.M.M., Šestan, N., Wildman, D.E.*, et al.* (2012). Prolonged myelination in human neocortical evolution. Proceedings of the National Academy of Sciences *109*, 16480-16485.

Ming, G.L., and Song, H. (2011). Adult neurogenesis in the mammalian brain: significant answers and significant questions. Neuron *70*, 687-702.

Moldovan, G.-L., Pfander, B., and Jentsch, S. (2007). PCNA, the Maestro of the Replication Fork. Cell *129*, 665-679.

Mullen, R.J., Buck, C.R., and Smith, A.M. (1992). NeuN, a neuronal specific nuclear protein in vertebrates. Development *116*, 201-211.

Nave, K.-A. (2010). Myelination and the trophic support of long axons. Nature Reviews Neuroscience *11*, 275-283.

Negoias, S., Croy, I., Gerber, J., Puschmann, S., Petrowski, K., Joraschky, P., and Hummel, T. (2010). Reduced olfactory bulb volume and olfactory sensitivity in patients with acute major depression. Neuroscience *169*, 415-421.

Nydal, R., and Lövseth, K. (1965). Distribution of Radiocarbon from Nuclear Tests. Nature *206*, 1029-1031.

Patani, R., Balaratnam, M., Vora, A., and Reynolds, R. (2007). Remyelination can be extensive in multiple sclerosis despite a long disease course. Neuropathology and Applied Neurobiology *33*, 277-287.

Patrikios, P., Stadelmann, C., Kutzelnigg, A., and Rauschka, H. (2006). Remyelination is extensive in a subset of multiple sclerosis patients. Brain 129, 3165-3172.

Penfield, W. (1924). Oligodendroglia and its relation to classical neuroglia. Brain *47*, 430-452.

Powers, B.E., Sellers, D.L., Lovelett, E.A., Cheung, W., Aalami, S.P., Zapertov, N., Maris, D.O., and Horner, P.J. (2013). Remyelination reporter reveals prolonged refinement of spontaneously regenerated myelin. Proceedings of the National Academy of Sciences *110*, 4075-4080.

Ransohoff, R.M., Hafler, D.A., and Lucchinetti, C.F. (2015). Multiple sclerosis-a quiet revolution. Nature reviews Neurology *11*, 134-142.

Rivers, L.E., Young, K.M., Rizzi, M., Jamen, F., Psachoulia, K., Wade, A., Kessaris, N., and Richardson, W.D. (2008). PDGFRA/NG2 glia generate myelinating oligodendrocytes and piriform projection neurons in adult mice. Nature Neuroscience *11*, 1392-1401.

Romanelli, E., Merkler, D., Mezydlo, A., Weil, M.-T., Weber, M.S., Nikić, I., Potz, S., Meinl, E., Matznick, F.E.H., Kreutzfeldt, M.*, et al.* (2016). Myelinosome formation represents an early stage of oligodendrocyte damage in multiple sclerosis and its animal model. Nature Communications *7*, 13275.

Ross, H.H., Levkoff, L.H., Marshall, G.P., Caldeira, M., Steindler, D.A., Reynolds, B.A., and Laywell, E.D. (2008). Bromodeoxyuridine Induces Senescence in Neural Stem and Progenitor Cells. Stem Cells *26*, 3218-3227.

Sakai, T., Mikami, A., Tomonaga, M., Matsui, M., Suzuki, J., Hamada, Y., Tanaka, M., Miyabe-Nishiwaki, T., Makishima, H., Nakatsukasa, M.*, et al.* (2011). Differential Prefrontal White Matter Development in Chimpanzees and Humans. Current Biology *21*, 1397-1402.

Salehpour, M., Forsgard, N., and Possnert, G. (2008). Accelerator mass spectrometry of small biological samples. Rapid Communications in Mass Spectrometry *22*, 3928-3934.

Salehpour, M., Håkansson, K., and Possnert, G. (2013). Accelerator mass spectrometry of ultra-small samples with applications in the biosciences. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms *294*, 97-103.

Salic, A., and Mitchison, T.J. (2008). A chemical method for fast and sensitive detection of DNA synthesis in vivo. Proceedings of the National Academy of Sciences *105*, 2415-2420.

Salzer, J.L., and Zalc, B. (2016). Myelination. Current biology *26,* R971-R975.

Sampaio-Baptista, C., Khrapitchev, A.A., Foxley, S., Schlagheck, T., Scholz, J., Jbabdi, S., DeLuca, G.C., Miller, K.L., Taylor, A., Thomas, N.*, et al.* (2013). Motor skill learning induces changes in white matter microstructure and myelination. The Journal of Neuroscience *33*, 19499-19503.

Sanai, N., Nguyen, T., Ihrie, R.A., Mirzadeh, Z., Tsai, H.H., Wong, M., Gupta, N., Berger, M.S., Huang, E., Garcia-Verdugo, J.M.*, et al.* (2011). Corridors of migrating neurons in the human brain and their decline during infancy. Nature *478*, 382-386.

Sanai, N., Tramontin, A.D., Quinones-Hinojosa, A., Barbaro, N.M., Gupta, N., Kunwar, S., Lawton, M.T., McDermott, M.W., Parsa, A.T., Manuel-Garcia Verdugo, J.*, et al.* (2004). Unique astrocyte ribbon in adult human brain contains neural stem cells but lacks chain migration. Nature *427*, 740-744.

Sánchez, M.M., Hearn, E.F., Do, D., Rilling, J.K., and Herndon, J.G. (1998). Differential rearing affects corpus callosum size and cognitive function of rhesus monkeys. Brain research *812*, 38-49.

Savas, J.N., Toyama, B.H., Xu, T., Yates, J.R., 3rd, and Hetzer, M.W. (2012). Extremely long-lived nuclear pore proteins in the rat brain. Science *335*, 942.

Schierbeek, A. (1953). The collected letters of Antoni van Leeuwenhoek. Antonie van Leeuwenhoek *19*, 181-188.

Schoenemann, P.T., Sheehan, M.J., and Glotzer, L.D. (2005). Prefrontal white matter volume is disproportionately larger in humans than in other primates. Nature neuroscience *8*, 242-252.

Scholz, J., Klein, M.C., Behrens, T.E., and Johansen-Berg, H. (2009). Training induces changes in white-matter architecture. Nature Neuroscience *12*, 1370-1371.

Scholzen, T., and Gerdes, J. (2000). The Ki-67 protein: From the known and the unknown. Journal of Cellular Physiology *182*, 311-322.

Seidl, A.H. (2014). Regulation of conduction time along axons. Neuroscience *276*, 126-134.

Seidl, A.H., Rubel, E.W., and Harris, D.M. (2010). Mechanisms for Adjusting Interaural Time Differences to Achieve Binaural Coincidence Detection. The Journal of Neuroscience *30*, 70-80.

Shields, S.A., Gilson, J.M., Blakemore, W.F., and Franklin, R.J.M. (1999). Remyelination occurs as extensively but more slowly in old rats compared to young rats following gliotoxin-induced CNS demyelination. Glia *28*, 77-83.

Sim, F.J., Zhao, C., Penderis, J., and Franklin, R.J.M. (2002). The Age-Related Decrease in CNS Remyelination Efficiency Is Attributable to an Impairment of Both Oligodendrocyte Progenitor Recruitment and Differentiation. The Journal of Neuroscience *22*, 2451-2459.

Smith, K.J., Blakemore, W.F., and McDonald, W.I. (1979). Central remyelination restores secure conduction. Nature *280*, 395-396.

Smith, R.S., and Koles, Z.J. (1970). Myelinated nerve fibers: computed effect of myelin thickness on conduction velocity. The American journal of physiology *219*, 1256-1258.

Snaidero, N., Mobius, W., Czopka, T., Hekking, L.H., Mathisen, C., Verkleij, D., Goebbels, S., Edgar, J., Merkler, D., Lyons, D.A.*, et al.* (2014). Myelin membrane wrapping of CNS axons by PI(3,4,5)P3-dependent polarized growth at the inner tongue. Cell *156*, 277-290.

Spalding, K., Bhardwaj, R.D., Buchholz, B., Druid, H., and Frisén, J. (2005). Retrospective birth dating of cells in humans. Cell *122*, 133-143.

Spalding, K.L., Arner, E., Westermark, P.O., Bernard, S., Buchholz, B.A., Bergmann, O., Blomqvist, L., Hoffstedt, J., Näslund, E., Britton, T.*, et al.* (2008). Dynamics of fat cell turnover in humans. Nature *453*, 783-787.

Spalding, K.L., Bergmann, O., Alkass, K., Bernard, S., Salehpour, M., Huttner, H.B., Bostrom, E., Westerlund, I., Vial, C., Buchholz, B.A.*, et al.* (2013). Dynamics of hippocampal neurogenesis in adult humans. Cell *153*, 1219-1227.

Stolt, C.C., Rehberg, S., Ader, M., Lommes, P., Riethmacher, D., Schachner, M., Bartsch, U., and Wegner, M. (2002). Terminal differentiation of myelin-forming oligodendrocytes depends on the transcription factor Sox10. Genes Dev *16*, 165-170.

Sturrock, R.R. (1980). Myelination of the mouse corpus callosum. Neuropathology and applied neurobiology *6*, 415-420.

Tang, Y.-Y., Lu, Q., Geng, X., Stein, E.A., Yang, Y., and Posner, M.I. (2010). Short-term meditation induces white matter changes in the anterior cingulate. Proceedings of the National Academy of Sciences *107*, 15649-15652.

Tasaki, I. (1939). The electro-saltatory transmission of the nerve impulse and the effect of narcosis upon the nerve fiber. American Journal of Physiology-Legacy Content *127*, 211-227.

Taupin, P. (2007). BrdU immunohistochemistry for studying adult neurogenesis: Paradigms, pitfalls, limitations, and validation. Brain Research Reviews *53*, 198-214.

Teicher, M.H., Dumont, N.L., Ito, Y., Vaituzis, C., Giedd, J.N., and Andersen, S.L. (2004). Childhood neglect is associated with reduced corpus callosum area. Biological psychiatry *56*, 80-85.

Tomassy, G., Berger, D.R., Chen, H.-H., Kasthuri, N., Hayworth, K.J., Vercelli, A., Seung, S.H., Lichtman, J.W., and Arlotta, P. (2014). Distinct Profiles of Myelin Distribution Along Single Axons of Pyramidal Neurons in the Neocortex. Science *344*, 319-324.

Toyama, B.H., Savas, J.N., Park, S.K., Harris, M.S., Ingolia, N.T., Yates, J.R., 3rd, and Hetzer, M.W. (2013). Identification of long-lived proteins reveals exceptional stability of essential cellular structures. Cell *154*, 971-982.

Tripathi, R.B., Rivers, L.E., Young, K.M., Jamen, F., and Richardson, W.D. (2010). NG2 Glia Generate New Oligodendrocytes But Few Astrocytes in a Murine Experimental Autoimmune Encephalomyelitis Model of Demyelinating Disease. The Journal of Neuroscience *30*, 16383-16390.

Vigano, F., Mobius, W., Gotz, M., and Dimou, L. (2013). Transplantation reveals regional differences in oligodendrocyte differentiation in the adult brain. Nature Neuroscience *16*, 1370-1372.

Virchow, R.L.K. (1863). Cellular pathology as based upon physiological and pathological histology ... / by Rudolf Virchow. Translated from the 2d ed. of the original by Frank Chance. With notes and numerous emendations, principally from MS. notes of the author (Philadelphia :: J. B. Lippincott).

Wang, C., Liu, F., Liu, Y.-Y., Zhao, C.-H., You, Y., Wang, L., Zhang, J., Wei, B., Ma, T., Zhang, Q*., et al.* (2011). Identification and characterization of neuroblasts in the subventricular zone and rostral migratory stream of the adult human brain. Cell Research *21*, 1534-1550.

Wang, S., Shultz, J.R., Burish, M.J., Harrison, K.H., Hof, P.R., Towns, L.C., Wagers, M.W., and Wyatt, K.D. (2008). Functional Trade-Offs in White Matter Axonal Scaling. The Journal of Neuroscience *28*, 4047-4056.

Winner, B., Kohl, Z., and Gage, F.H. (2011). Neurodegenerative disease and adult neurogenesis. European Journal of Neuroscience *33*, 1139-1151.

Wu, L., Williams, A., Delaney, A., Sherman, D.L., and Brophy, P.J. (2012). Increasing Internodal Distance in Myelinated Nerves Accelerates Nerve Conduction to a Flat Maximum. Current Biology *22*, 1957-1961.

Yakovlev PI, Lecours A-R. The myelogenetic cycles of regional maturation of the brain. In: Minkowski A, editor. Regional development of the brain in early life. Blackwell Scientific; Oxford, UK: 1967. pp. 3–70

Young, K.M., Psachoulia, K., Tripathi, R.B., Dunn, S.-J.J., Cossell, L., Attwell, D., Tohyama, K., and Richardson, W.D. (2013). Oligodendrocyte dynamics in the healthy adult CNS: evidence for myelin remodeling. Neuron *77*, 873-885.

Zawadzka, M., Rivers, L.E., Fancy, S.P.J., Zhao, C., Tripathi, R., Jamen, F., Young, K., Goncharevich, A., Pohl, H., Rizzi, M*., et al.* (2010). CNS-Resident Glial Progenitor/Stem Cells Produce Schwann Cells as well as Oligodendrocytes during Repair of CNS Demyelination. Cell Stem Cell *6*, 578-590.

Zhu, X., Hill, R.A., Dietrich, D., Komitova, M., Suzuki, R., and Nishiyama, A. (2011). Age-dependent fate and lineage restriction of single NG2 cells. Development *138*, 745-753.