General Introduction
1. **Glia: the “other” class of brain cells**

The central nervous system (CNS) is composed of many different types of cells. With the help of elaborate staining procedures [1], and the development of cell electrophysiology analysis at the beginning of the 20th century, brain cells were initially subdivided into two main categories, i.e., neurons and glial cells. It was discovered that neurons are electrically excitable, and communicate with each other via the generation of action potentials and using specialized neuronal substructures known as synapses. Further research on neurons indicated that these characteristics form the basis for how these cells sense, integrate and react to information arising outside the brain. Thereby neurons play key-functions in healthy brain functioning, driving physiology and behavior. Glial cells were not found to respond to electrophysiological stimulation, and therefore considered no more than the mere cement keeping the neurons in place and sticking these together. Indeed, glia literally means glue in Greek. The field of neurosciences thus further developed focusing its attention on neurons and neuronal functions, whereas glial cells were deemed to be of lesser importance.

During the second half of the 20th century, newly developed immunohistological and imaging techniques led to changes in the perception of glial cells, and revealed that they are involved in many more different brain functions than was previously thought. It became apparent that the notion of glia as mere housekeeping cells is too simplistic, and that glial cells are as important as neurons for the brain to properly function. Furthermore, the group of CNS glial cells was further subdivided into two main groups: the microglial and macroglial cells, with the latter consisting of oligodendrocytes and astrocytes. Oligodendrocyte and astrocyte lineage cells are derived from neural stem cells, whereas microglia originate from the immune system [2]. Further sub-divisions and categorizations of glial cell-types can be made, such as the myelinating Schwann cells in the peripheral nervous system (PNS), and the NG2+ cells in the CNS [2], but these are not the focus of the current thesis. Here, we will
focus mostly on the group of CNS macroglial cells. For more information on microglia, extended categories and/or glial cells outside the CNS, I would like to refer to the reviews of Eroglu & Barres [2], Ransohoff & Cardona [3], Graeber [4], Jessen & Mirsky [5], and Sherman & Brophy [6].

1.1. **Oligodendrocytes: more than merely insulating cells?**

Oligodendrocytes have long been recognized for having a clear and specific function in myelination of the CNS. Myelin is a specialized cellular compartment of oligodendrocytes that spirally wrap membrane extensions around axons. Myelin has isolating properties, and by increasing conduction velocity, it plays an important role in the acceleration of signal transduction [6]. The myelin sheath and the way it interacts with neuronal axons comprises a complex biological system, in which different compartments each play different but valuable roles (figure 1). In motor and sensory functioning often large distances have to be bridged, for example between the CNS and cells in peripheral limbs. In these cases, the importance of myelination and high conduction velocity is very evident. In diseases that affect CNS myelination, such as multiple sclerosis, sensory and motor deficits represent core symptoms, and this has attracted the bulk of attention in studies on glial cells and myelin.

The relevance of myelin in cognition and behavior is less tangible. However, myelin is now attracting new interest as an unexpected contributor to cognitive performance and to a wide range of psychiatric disorders [7]. Numerous studies have emerged that point at conduction velocity and processing speed as predictors of general intelligence [8, 9], or that report correlations between processing speed and cognitive impairments for patients with multiple sclerosis [10]. With the development of diffusion tensor imaging (DTI), it has become possible to visualize alterations in myelin tracts [11]. Myelin abnormalities have subsequently been implicated in a wide variety of mental disorders such as schizophrenia, autism, depression, drug addiction and Alzheimer's disease [12-18]. These findings have been supported by genome-
wide expression analyses, showing deregulation of myelin and oligodendrocyte genes in postmortem tissue from schizophrenic patients, patients with bipolar disorder and patients with major depression disorder [7, 19-22]. Finally, studies with mice have shown that manipulation of oligodendrocyte genes that regulate glial development and myelination cause behavioral abnormalities that might relate to schizophrenia [7, 23].

Figure 1. Schematic drawing showing the different components of the CNS myelin sheath. “Compact myelin” makes up the largest part of the internodal region and the myelin sheath. Here, up to 150 layers of oligodendrocyte extensions are tightly wrapped around the axon. The primary function of proteins in the compact myelin is to stabilize the apposed myelin membranes. This part of the myelin sheath includes myelin basic protein (MBP), proteolipid protein (PLP), myelin-associated oligodendrocytic basic protein (MOBP), and myelin and lymphocyte protein (MAL). The “radial component” is a structural feature specific for CNS myelin. It is formed by intra-lamellar strands that span the myelin sheath. This tight junctional array contains claudin-11 (CLDN11), marks the border between compact and non-compact myelin, and may act as a diffusion barrier between these myelin subdomains. The myelin layers terminate in the “paranodal” region, where the “non-compact myelin” is located. Here, the myelin sheath is anchored to the axon. The non-compact regions of myelin contain 20,30-cyclic nucleotide 30-phosphodiesterase (CNP), myelin-associated glycoprotein (MAG), myelin oligodendrocyte glycoprotein (MOG), and the 155 kDa isoform of neurofascin (NF155). Neuronal cell adhesion molecule (NCAM) consists of different isoforms that, among other locations and functions, are located at the axon and at myelin membranes and play roles in adhesion of the myelin to the axon. The “node of Ranvier” is the gap between two sections of myelin where the axon is exposed. Here, most ion channels are located.

Interestingly, myelination in the human brain continues well into the third decade of life. This coincides with the same period when human cerebral cortex undergoes massive remodeling of synaptic connections, which are understood to modify the
brain according to experience. This raises the question whether myelin might also play a role in plastic processes influenced by experience [7]. Indeed, numerous studies in animals and humans have shown correlations between experience and oligodendrocyte numbers and/or white matter structure [7, 24-31]. In addition, several studies have shown that interference or stimulation of neuronal activity in mixed cell cultures can affect myelination or the expression of myelination-regulating molecules, such as cell adhesion molecule L1 (L1-CAM) and leukemia inhibitory factor (LIF) [32-35]. Furthermore, specific myelin proteins, such as neurite outgrowth inhibitor A (NOGO-A), myelin-associated glycoprotein (MAG) and oligodendrocyte-myelin glycoprotein (OMgp) can directly influence the formation of synaptic circuits during development, by inhibiting sprouting of axons and influencing critical periods of synaptic plasticity [7, 36-38]. An interesting theory poses that myelin might even be able to adapt the speed of neuronal signaling in response to environmental demands [7]. The importance of temporal coincidence of firing onto postsynaptic neurons for processes of synaptic plasticity has been well known and broadly accepted. Recently, it has been suggested that myelin could play an important role in this process by actively regulating the speed of transmission in axons of different lengths, and modulation the synchronous arrival in post-synaptic targets [7].

Taken together, the above-described studies provide new perspectives on the roles of oligodendrocytes and myelin in brain functioning. Myelin is modifiable by experience, and might affect information processing in the brain by regulation of conduction velocity and synchrony of impulse conduction. Slowed or desynchronized impulse conduction between distant brain regions might underlie and contribute to a wide range of cognitive abilities or psychiatric diseases. Currently, it is not clear whether the observed changes in oligodendrocytes or myelin under those circumstances may represent primary glial-genetic contributions, or are merely secondary responses to alterations or differences in neuronal functioning. Further research into genetic and molecular mechanisms will help to advance our
understanding of the contribution of glial factors in psychiatric and neurological diseases.

1.2. Astrocytes: from support to active participants in communication and plasticity

Even though astrocytes are the most abundant cell-type in the brain, their roles in CNS functioning have only been appreciated recently. A wide range of brain functions were discovered, which made them viewed upon as cells that provided the support to ensure optimal neuronal functioning in the CNS [39]. These functions include regulation of blood flow, the uptake and delivery of nutrients from the blood-brain barrier (BBB) to neurons, involvement in brain metabolic processes, and immunological functioning during inflammatory reactions and injuries [2, 39, 40].

In the late 1980s it was recognized that astrocytes express voltage-gated channels, neurotransmitter receptors [39], and are closely associated with presynaptic and postsynaptic terminals [40-42]. It was also found that astrocyte differentiation in the mammalian brain occurs simultaneously with the massive synaptogenesis in the early postnatal period [2, 40]. These discoveries led to the idea that astrocytes might not only support, but also actively participate in CNS communication and plasticity processes [39]. Noteworthy, a single rodent astrocyte can associate with multiple neurons and can contact up to thousands of synapses [43, 44]. By coordinating activity among sets of neurons, astrocytes might thus greatly contribute to fine-tuning of neuronal connections in the brain. As a result of these findings, their previous “support” functions are now viewed in a much more dynamic perspective. For instance, by having contact with capillaries as well as with multiple synapses, astrocytes may supply neurons with nutrients in accordance with the intensity of their synaptic activity [40].
These discoveries also led to newly appreciated functions involving interactions between astrocytes and neuronal synapses. Recent studies in rodents have shown that astrocytes can use both secreted and contact-mediated signals to control synapse formation. For instance, astrocytes express and secrete factors that control synapse formation (e.g. APOE-bound cholesterol, thrombospondins, hevin and SPARC), synapse maturation (Glypicans), synaptic pruning (TGF-beta), and spine formation (Ephrin A3) [2, 39, 45-47]. In addition, astrocytes can release a wide range of so-called gliotransmitters, including glutamate, GABA, acetylcholine, noradrenaline, dopamine, ATP, nitric oxide, BDNF, and D-serine [2, 39, 48], that are believed to play important roles in a variety of synaptic responses, such as activity-dependent modulation of synaptic excitability, neuronal transmission and synchronization of synaptic events [42, 48, 49]. These observations confirm the tripartite nature of many synapses [50] in which astrocytes represent a third element of the synapse signal-integration unit [39, 48]. Astrocytes are now thus viewed as ‘excitable’ cells in the sense that, when activated by internal or external signals, they deliver specific messages to neighboring cells. In contrast to neurons, their excitation cannot be revealed by electrophysiology, but by determination of Ca\(^{2+}\) transients and oscillations [39, 51].

Importantly, astrocyte excitation and gliotransmission have been shown to be involved in various pathological brain conditions. Spontaneous Ca\(^{2+}\) oscillations in astrocytes are abolished after traumatic mechanical insult, but are stimulated in certain forms of epileptic activity [39]. After ischemic insult, hippocampal astrocytes express NMDARs that they do not express under normal circumstances. Subsequently, they generate abnormal calcium signals that might compensate for inactivity of NMDARs in neurons, and that help to reduce degeneration of afferent fibers [12]. In response to acute glial inflammation, the changes might be less beneficiary, as astrocytic glutamate release is amplified which causes a neurotoxic process, known as excitotoxicity [39]. Astrocytes might also play key roles in the pathology of dementias as observed in AIDS and Alzheimer’s disease [39], and have
been linked to psychiatric diseases as schizophrenia and mood disorders [42, 52-56]. Nevertheless, little is known about the underlying mechanisms by which astrocytes are involved in these diseases. Nor is it known whether their involvement is a result of neuronal dysfunktioning, or whether they can primarily contribute to these disease phenotypes.

2. Lipids in the CNS: special roles for glial cells

The CNS is shielded from lipids in the circulation by the BBB, and is therefore thought to be highly autonomous in lipid metabolism [40, 57, 58]. Lipid metabolism may be of particular importance in the CNS, as the brain has a very high concentration of lipids, second only to adipose tissue [58]. A crucial role of lipids in brain physiology and signaling is demonstrated by the many neurological disorders, including bipolar disorder, schizophrenia, Alzheimer’s and Parkinson’s, Niemann-Pick and Huntington disease, that involve deregulated lipid metabolism [58]. Interestingly, glial cells, and not neurons, are the key players in synthesis and metabolism of brain lipids, and thus represent important targets for lipid-mediated brain disorders, as will be further described below.

2.1. Oligodendrocytes and lipids

The insulating capabilities of myelin are largely dependent on the myelin thickness, as well as on its high and specialized lipid content [59, 60]. In contrast to other membranes, lipids account for at least 70% of the dry weight of myelin membranes [59]. The importance of lipids in myelin synthesis has been emphasized by research in rodents, showing that expansion of the myelin membrane is highly correlated with accumulation of cholesterol and lipids in the CNS [59, 61, 62], as well as with transcriptional expression of lipid enzymes [59, 61]. Even though the myelin membrane does not contain truly myelin-specific lipids, it has a very peculiar lipid
composition, characterized by very high levels of cholesterol, glycosphingolipids, plasmalogens and long-chain fatty acids [59, 60, 63].

**Cholesterol** is one of the most important regulators of lipid organization in the membrane [59]. Via interactions with polar heads and fatty acid tails of other lipids, cholesterol can affect biophysical properties of the membrane like fluidity, diffusion of membrane proteins, bending capacity of the membrane, and the permeability of polar molecules including ions [59, 64-67]. Ultimately, these properties can all contribute to the insulating function of the myelin membrane [59, 68]. In diseases as Smith-Lemli-Opitz syndrome, in which cholesterol metabolism is affected, white matter defects, cognitive impairment and behavioral deficits represent an important part of the syndrome [59]. The anionic glycol group in glycoprophingolipids may provide an electronic shielding on the outside of the myelin membrane, and thereby contribute to the insulating properties of the myelin membrane [59]. Moreover, glycosphingolipids can be solubilized by cholesterol, while both lipids are predominantly present in the outer leaflets of the myelin membrane. This suggests that glycosphingolipids and cholesterol might regulate curving and fluidity of myelin membranes, which could be of particular importance for the formation of paranodal loop structures [59, 69]. Indeed, knockout mice that lack glycosphingolipids are characterized by a nodal phenotype. Besides, they show increased membrane fluidity and increased ion permeability, which may disrupt saltatory conduction [59, 70-72].

Plasmalogens are a sub-class of ether phospholipids [59]. The roles of plasmalogens in membranes are not well known, but they are thought to play a role in membrane fusion processes and membrane dynamics. They can increase membrane fluidity and stimulate the formation of nonbilayer lipid structures when present in high concentrations [59, 73]. Accordingly, plasmalogens could be involved in myelin membrane formation or maintenance by potentiating membrane dynamics [59, 74]. Finally, very long-chain fatty acids (VLCFAs) in the CNS are specifically enriched in myelin, whereas the brain is enriched in long polyunsaturated fatty acids (PUFAs) like DHA [59, 75]. Phospholipids containing VLCFA may decrease membrane fluidity, give
a higher degree of fatty acid ordering in the membrane, and contribute to electric insulation of the axon [59].

Taken together, many of the myelin-enriched lipids thus play important roles in the insulating properties of the myelin, and thereby in the saltatory conduction of ensheathed axons [59]. In addition, lipids are also important for proper sorting and anchoring of myelin proteins to the myelin sheath [59, 64]. Both conduction velocity and myelin structure are clearly affected in many lipid metabolic disorders, indicating that at least part of the damage might be caused by direct influences of the compromised lipid metabolism [59]. Accordingly, a mouse model in which the cholesterol precursor squalene synthase (SQS) was knocked-out specifically in oligodendrocytes, showed that the availability of cholesterol is an essential, rate-limiting factor for myelin membrane growth in the CNS. Unexpectedly however, myelination seemed to recover over a longer period of time [68]. In a peripheral model, a Schwann cell-specific knockout mouse for the lipid regulating protein SCAP was created [76]. SCAP is a sterol responsive element binding protein (SREBP) cleavage activation protein that is needed for the posttranslational activation of SREBP transcription factors. Activated SREBPs translocate to the nucleus, where they induce genes containing sterol responsive elements [77]. Hence, inactivating SCAP affects cholesterol and lipid homeostasis by interfering at the transcriptional level with their biosynthesis and uptake. It was shown with this model that also in the peripheral nervous system (PNS), cholesterol is rate limiting for myelination, and that rescue of myelination takes place over time [76]. Taken together, these studies show that both oligodendrocytes and Schwann cells are able to take up cholesterol from their surroundings and use these to enrich myelin membranes [59]. Further investigation of the cellular and molecular processes that underlie myelin membrane synthesis is needed for further understanding and development of treatment of CNS diseases that are associated with defective lipid metabolism.
2.2. Astrocytes and lipids

Astrocytes are capable of synthesizing many different lipid classes, including mono-unsaturated fatty acids (MUFAs), PUFAs and sterols [40, 78]. Moreover, they might act as important lipid providers to surrounding cells in the CNS. Astrocytes express the desaturases D5D and D6D [79] and unlike neurons, they are very active in desaturation and elongation of essential fatty acids (EFAs) into PUFAs [80]. In line with this, it was found that neurons in different brain regions can take-up astrocyte-derived PUFAs and incorporate them into phospholipids [78].

The supply of lipids from astrocytes to neurons has been proposed to play important roles in neurodevelopmental processes, such as in neurite outgrowth, synaptogenesis and subsequent regulation of synaptic transmission [40, 81]. For instance, astrocytes synthesize and release oleic acid, which is enriched in neuronal growth cones, and can induce neuronal differentiation in vitro [82]. In addition, astrocyte-derived cholesterol has been found to represent a major signal for promotion of synaptogenesis in vitro [81, 83, 84]. More speculative is the idea that astrocyte derived lipids might also regulate synaptic plasticity in the adult brain [40]. In accordance with this idea, it has been shown that the low-density lipoprotein (LDL) receptor-related protein (LRP) plays an active role in synaptic plasticity in the mouse hippocampus [85]. Moreover, pharmacological inhibition of cholesterol synthesis in rat hippocampal slices inhibits synaptic plasticity [86]. We recently provided the first in vivo evidence that astrocyte lipid metabolism is required for proper brain function [87]. We showed that mice with obliterated astrocyte lipid synthesis develop severe motor dysfunction and die prematurely [87].

In addition to the view of astrocytes as important lipid providers to neurons, there are indications that astrocytic lipid metabolism may also play important roles in oligodendrocyte functioning and the production and synthesis of myelin. As described in the above paragraph on oligodendrocytes and lipids, gene deletions in Schwann cells and oligodendrocytes affecting lipid metabolism, have been shown to
affect myelination [59, 68, 76]. Importantly, it was shown in both models that hypomyelination returned to close to normal levels after a few months, suggesting a rescue of myelination by uptake of extracellular lipids. In particular astrocytes, which are unlike neurons highly active in lipid metabolism and secretion, are good candidates to supply such extracellular lipids to oligodendrocytes for CNS myelination. Indeed, astrocytes are known to promote myelination of dorsal root ganglion neurons in response to electrical activity by releasing the cytokine LIF [33]. Astrocytes, but not olfactory ensheating cells or Schwann cells, support myelination of dissociated spinal cord cultures [88]. And finally, it was shown that astrocytes, when co-cultured with purified retinal ganglion neurons and oligodendrocyte precursors (OPC), specifically enhance myelin growth rather than affecting OPC proliferation or survival [89]. These studies showed that astrocytes are able to support myelination in vitro. However, whether they actively support myelination in vivo, and the mechanisms behind this, remain to be determined.

3. Aim & Scope of this thesis

Glial cells play important roles in CNS functioning, and during the last decade their contribution to normal brain functioning and pathological conditions has been increasingly acknowledged. For both oligodendrocytes and astrocytes, their attributed roles in the brain have increased from merely giving structural neuronal support, to the regulation of processes ranging from neurodevelopment and correct brain assembly, to neuroplasticity and maintenance of brain connectivity circuits in the adult brain. Despite this increasing awareness of the importance of glial cells in regulation of brain functions in health and disease, much knowledge is lacking on the genetic contribution of glial cells to neurological disorders, in particular polygenic disorders such as psychiatric diseases. Importantly, both oligodendrocytes and astrocytes can contact multiple neurons, and as such are able to coordinate neuronal communication and activity among sets of neurons. Especially oligodendrocytes
might play important roles in integration of information from multiple brain regions, via influencing conduction velocity of axons over large distances in the brain. As lipids represent important constituent of the myelin membrane sheath, these might also play important roles in myelin-mediated processes. This thesis aims to gain more insights into the roles of oligodendrocytes and astrocytes in CNS connectivity and psychiatric disease. We focused on glial genetic variations that may contribute to variation in behavioral functioning or illness (Chapters 2, 3 and 4), on CNS connectivity (Chapters 4 and 5), and on the role of glial lipid metabolism in myelination (Chapter 5). A summary of this approach is shown in figure 2. This multidisciplinary approach to investigate contributions of glial genetics in humans to polygenic mental disorders, and in mice to behavior and CNS connectivity, identified specific glial functions that might underlie primary processes in CNS diseases and disorders.

**Figure 2. Outline of this thesis.** Shown is the outline of this thesis, for which we started with a bioinformatics approach to investigate associations between glial genetic variations and human psychiatric disorders. For this purpose, expert curated glial gene sets were generated based on published microarray gene expression data. Via functional gene set analysis, the combined effects of genetic variants in glial type-specific genes for association with schizophrenia (Chapter 2) and Tourette Syndrome (Chapter 3) were investigated. In chapter 4 we moved on to a mouse model to investigate biochemical and functional effects of genetic variation on myelination and myelin-associated connectivity and behavior in BXD recombinant inbred (RI) strains. Finally, in chapter 5, the role of oligodendrocyte-astrocyte interactions in lipid biosynthesis for brain myelination and connectivity was investigated using transgenic mouse lines generated by cre-mediated recombination of SCAP in astrocytes, oligodendrocytes, or both.
References


Chapter 1: General Introduction


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