CHAPTER 6

General discussion
1. Loss of SREBP-dependent lipid metabolism in astrocytes leads to paroxysmal dyskinesia and premature death

Our observations on GFAP-SCAP mice show that deletion of SCAP in astrocytes leads to the development of paroxysmal dyskinesia, unbalance and tremors, and to premature death. Paroxysmal dyskinesias (PxD) are a heterogeneous group of motor deficits that display as inducible episodes of dystonia accompanied by choreoathetosis (irregular and uncontrolled involuntary movements) (Fahn 2002). Familial cases of PxD have been studied for which a variety of gene mutations have been identified that are generally linked to perturbed synaptic function (Fahn 1989; Chen et al. 2011). In contrast to these familial cases, also referred to as primary PxD, the PxD observed in GFAP-SCAP mutants appears to be rather secondary and most likely due to malformations of the brain (chapter 3). Important in this respect are the observations that GFAP-SCAP mutant mice have severe CNS hypomyelination (chapter 4) and impaired synaptic function (chapter 5), which both may contribute to PxD in GFAP-SCAP mutants, as I will discuss below.

Myelin enhances action potential propagation (conduction velocity), and may modulate action potential synchronization (Hartline and Colman 2007), and as such is essential for interconnection of different brain regions as well as for correct motor control via axonal innervations of muscles. Accordingly, motor control is often compromised in diseases affecting CNS myelin, such as in Pelizaeus-Merzbacher disease (PMD) (Thomson et al. 1997), vanishing white matter disorder (van der Knaap et al. 2002), and in Multiple Sclerosis (Verheul and Tyssen 1990; Florczak and Kozubski 2003). Similarly, defects in myelination and motor dysfunction are observed in many lipid metabolic diseases (Chrast et al. 2011), e.g., in Niemann Pick type C disease (Walterfang et al. 2011; Xiong et al. 2012) and Smith-Lemli-Opitz (Nwokoro et al. 2001; Tierney et al. 2001). In some animal models for demyelinating diseases (e.g., PMD, ALD) myelin deficits are causing motor deficits that are apparent as paralysis and/or epilepsy (Thomson et al. 1997; Kassmann et al. 2007). These are not observed in GFAP-SCAP mutants, since mutants do not develop paralysis even at advanced disease stages and do not present any post-ictal periods as observed for epileptic attacks. Although misdiagnosis between epilepsy and
paroxysmal dyskinesia has been common in humans, it is currently accepted that both movement disorders can be clinically differentiated (Guerrini 2001; Guerrini et al. 2002). However, co-occurrence of PxD and epilepsy has also been observed and it has been proposed that a common pathophysiological abnormality can be differentially expressed in the cortex and the basal ganglia, leading to either PxD or epilepsy (Guerrini et al. 2002). PxD has been reported as a rare initial manifestation of multiple sclerosis (Verheul and Tyssen 1990; Florczak and Kozubski 2003) and possibly linked to premature loss of myelin. The reason that we did observe PxD but neither epilepsia nor paralysis may depend on the degree of myelination present in different mouse models and the anatomical localization of the defects. The main observable feature of paroxysmal dyskinesias is a disproportionate contraction of multiple muscles of the body, when the subject attempts to initiate a voluntary movement. This suggests that synchronization of the flexing and contraction of different muscles is compromised. Whether and how this is explained by hypomyelination remains to be determined, but two main mechanisms may be responsible for this. First, hypomyelination may impair conduction velocity and thereby the synchronisation of different axonal outputs towards their targets. As such, timely regulated excitation and inhibition are lost, which on their turn cause too strong and discordant responses when initializing a movement. Second, the loss of myelin around axons may lead to poor axonal isolation causing shortcuts between non-myelinated axons yielding incorrect wiring and activation of wrong targets.

Importantly, like in other movement disorders, PxD could also be a cause of neuronal dysfunction. In many neurodegenerative diseases, the loss of axons and neurons leads to impaired motor behaviour. For instance, in Parkinson disease, the loss of neurons in the substantia nigra leads to uncontrolled tremors. Also, in Huntington disease, which is mainly characterized by choreatic movements, the neuropathological hallmark is atrophy and neuronal loss in various brain regions such as the striatum, and in other parts of the basal ganglia, as well as many other brain regions. In SCAP mutants we do not observe neuronal loss at the studied time points (3 weeks old mice), however we do observe axonal swellings that can lead, at later time points, to neuronal degeneration and death. Besides that myelin is required for proper conduction velocity, it also has a protective role for axons (Kassmann and Nave 2008; Nave and Trapp 2008). Accordingly, myelin deficits lead to axonal
swellings, inflammation, axonal degeneration and neuronal death (Griffiths et al. 1998). In SCAP mutants I have detected axonal swellings in the axons of Purkinje cells in the cerebellum, which may be in part responsible for the observed motor deficits. Whether, neuronal loss occurs in older animals remains to be determined.

In addition, PxD in GFAP-SCAP mutants may be caused by aberrant synapse morphology and function. For instance, we found deficient synaptic transmission in the hippocampus (Chapter 5), and even though the hippocampus is not known for playing a role in these types of movement disorders, it is likely that synaptic transmission is also impaired in other brain regions. This includes the cerebellum for which we observed strong atrophy in GFAP-SCAP mutants (Chapter 3), and may include other regions involved in motor behaviour such as the basal ganglia. Interestingly, both the cerebellum and the basal ganglia have been proposed to be an anatomical substrate of PxD and together form the motor network that is responsible for the generation and also the severity of dystonic attacks in human cases and also in animal models (Sandoval-Romero and Felix-Grijalva 2003; Devanagondi et al. 2007; Neychev et al. 2008).

PxD attacks in SCAP mutants take place when animals are inactive and aim to initiate a movement. This is in line with a possible involvement of the basal ganglia and cerebellum (Shmuelof and Krakauer 2011). While the basal ganglia of GFAP-SCAP mutant mice remain to be analyzed in detail, we did find strong morphological changes in the cerebellum of SCAP-GFAP mutants, including thinner molecular and granular cell layers as well as aberrant Bergmann glia alignment. Bergmann glia act as directors for Purkinje cell neurite outgrowth through the molecular layer of the cerebellum (Lordkipanidze and Dunaevsky 2005) and influence the number of synapses on Purkinje cell dendrites (Lippman Bell et al. 2010). Moreover, Bergmann glia have been suggested to act as a third partner of synapses in the molecular layers between the presynaptic fibers and the postsynaptic dendrites of the Purkinje cells (Reichenbach et al. 2010). Through this configuration, the so called tripartite synapse, Bergmann glia modulate synaptic transmission, similar as astrocytes do in other parts of the brain (Saab et al. 2012). Primary PxD has been linked to channelopathies as well as deficient vesicular fusion (Margari et al. 2005; Chen et al. 2011) suggesting a prominent role for synaptic transmission, at least in primary PxD. The observed aberrant morphology of Bergmann glia in GFAP-SCAP mutants may affect Purkinje
cell function through deficient modulation of synaptic activity, leading to impaired synaptic transmission as observed in primary PxD cases (Margari et al. 2005).

GFAP-SCAP mutants show premature death around 12 weeks of age for some animals. Notably, all animals get ill and death is preceded by more pronounced tremors, unbalance and by PxD attacks that become more frequent and severe. This may suggest that PxD attacks are related to the cause of premature death, and one could speculate that if the severity and duration of the attacks increase above a certain threshold, it will lead to death. How PxD and motor dysfunction in SCAP mutant may cause premature death is largely unknown. Because the attacks in GFAP-SCAP mutants involve exaggerated muscle contraction, a hypercontraction of the respiratory muscles may lead to asphyxia and subsequently to death. Asphyxia and subsequent death has been reported for other mutant mouse lines with myelin defects (Kassmann et al. 2007). Finally, our observation that a high fat diet rescues premature death, reduces the PxD attacks and also improves the myelin deficits, suggest a causal connection between these phenotypes. The high fat diet did not restore brain size and reduce anxiety of GFAP-SCAP mutants, arguing for another pathophysiological mechanism underlying those phenotypes.

2. Myelin membrane synthesis relies on endogenous lipids synthesis and extracellular lipid uptake; implications for myelination and remyelination

We and others have demonstrated in vivo, using genetically modified mice, that myelinating cells rely on self-produced lipids for the synthesis of myelin, in a timely regulated way (Saher et al. 2005; Verheijen et al. 2009). However, as we have found for P0-SCAP KO mice (chapter 2, (Verheijen et al. 2009), and others for the CNP-squalene synthase KO mice (Saher et al. 2005), defects in endogenous lipid synthesis in Schwann cells and oligodendrocytes can partially be overcome by extracellular lipid uptake. Moreover, we have shown in vitro that also wild-type Schwann cells form less myelin when levels of lipids in the medium are decreased (Verheijen et al. 2009). The contribution of extracellular lipids to normal myelination by Schwann cells in vivo needs to be determined, but it may be more important than previously expected. In the case of PO-SCAP mutant mice, the deletion of SCAP is specific to myelinating Schwann cells, leaving the possibility that these cells take up lipids from neighbouring cells in the nerves, such as non-myelinating Schwann cells or
fibroblasts. The mechanism by which Schwann cells take up extracellular lipids remains to be determined. It has previously been shown that the expression of the LDL receptor in Schwann cells is increased during myelination (Verheijen et al. 2003). However, myelination is possible in the absence of the LDL receptor (Goodrum et al. 2000). Therefore, the use of LDL receptors by Schwann cells may be facultative, possibly dependent on the need for exogenous lipids. Importantly, the expression of the LDL receptor is dependent on SREBP2 activity (Horton et al. 2003), which makes a possibly role for LDL receptor-mediated uptake of lipids in SCAP mutants very unlikely, although expression analysis for the LDL receptor as well as other lipid receptors in PO-SCAP mutant Schwann cells will be necessary to conclude on this. In myelin of PO-SCAP mutant mice the balance between monounsaturated and polyunsaturated fatty acids (PUFA) is shifted towards PUFA (Verheijen et al. 2009). These PUFA are essential fatty acids that must be acquired by the Schwann cells by uptake from the circulation and as such shows the contribution of dietary lipids. Our observation that SCAP mutant Schwann cells benefit from extracellular lipid uptake, is an argument to test the application and optimization of a lipid-enriched diet to improve hypomyelination in P0-SCAP mutant mice, which finally may help to improve myelination in patients with congenital hypomyelination.

Saher et al., previously suggested that CNS hypomyelination in mice of which oligodendrocytes lack cholesterol synthesis (Saher et al. 2005), may be overcome by extracellular uptake, but that feeding of the mutant animals with a cholesterol enriched diet did not improve hypomyelination. These results are different from what we have shown using SCAP mutants. In GFAP-SCAP mutants, oligodendrocytes are able to produce their own lipids, however, they are affected by deficient lipid synthesis in astrocytes, and through astrocytes benefit from dietary lipids (Chapter 4). Therefore, similarly to the PNS, the contribution of extracellular lipid uptake for normal myelination may be larger than what was proposed previously. In line with this, Saher et al., more recently have shown that PMD mice benefit from a high cholesterol enriched diet, even though in these mice cholesterol deficit is not the principal cause of demyelination (Saher et al. 2012). It was recently shown by Watkins et al. that astrocytes do not affect oligodendrocyte proliferation and survival but that they are necessary for the final steps in myelination, resulting in thicker myelin sheaths when astrocytes are present, at least in vitro (Watkins et al. 2008).
Astrocyte independent | Astrocyte dependent
---|---
Ensheathment | Wrapping | Growing

Wild-type

GFAP-SCAP

GFAP-SCAP

HFD

Oligodendrocyte

Astrocyte

Big axon | Small axon | Endogenous lipids | Diet lipids

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Based on this and our observations discussed below we propose that this effect of astrocytes in oligodendrocyte wrapping is mediated by lipid secretion from astrocytes, that leads to an increase of lipid availability and fast incorporation in the growing myelin sheath (Figure 1).

While we found normal levels for myelin proteins and cholesterol, as well as normal numbers of oligodendrocyte precursors in 2 weeks old GFAP-SCAP mutants, reduced levels of cholesterol and myelin proteins were found in mutant adult brains. The levels of myelin proteins in adult GFAP-SCAP mutants resembled the levels in 2 weeks old wild-type animals, whereas for wild-type animals these levels are largely increasing towards adulthood. Therefore, we conclude that in GFAP-SCAP mutants, oligodendrocytes are able to produce an amount of lipids that is sufficient for myelin membrane growth until the age of 2 weeks, but that oligodendrocytes are becoming dependent on astrocyte-derived lipids for further growth of myelin, that normally takes place until adulthood. Because cholesterol is rate limiting for myelin formation (Saher et al. 2011), it is not surprising that in the absence of lipids, among which cholesterol, myelin protein levels are also reduced.

Interestingly, we find that in particular small diameter axons are devoid of myelin. This is in line with observations that large diameter axons are the first to be myelinated (Nave and Trapp 2008). We propose that during the first postnatal weeks endogenous lipid levels in oligodendrocytes are sufficient for myelin membrane synthesis around large diameter axons, while the subsequent full myelination of these large axons, as well as the myelination of small calibre axons, requires lipid supply from astrocytes. Importantly, feeding the mutants with a cholesterol- and oleic acid-enriched diet led to increase in myelination, in particular, small diameter axons did benefit from this treatment. This indicates that lipids, with elevated circulation levels, can reach the brain and are incorporated in the growing myelin membrane (Figure 1).

**Figure 1.** Proposed model for the role of astrocyte- and dietary-derived lipids in CNS myelination. Oligodendrocytes produce enough lipids for initial steps of myelination, including ensheathment. For wrapping and myelin sheath volume growth, oligodendrocytes are dependent on astrocyte-derived lipids. In GFAP-SCAP mutants, astrocyte-derived lipids are absent, leading to thinner myelin in large-diameter axons, and no myeline in small-diameter axons. When GFAP-SCAP mutants are fed with a high fat diet (HFD) a larger number of axons are myelinated.
Taken together, these results show that both in the PNS and CNS the requirement of extracellular lipids for myelination is more important than previously thought. This may have important implications for the understanding of myelin-associated diseases but also for the development of strategies to treat myelin defects by enhancing extracellular lipid uptake.

3. Use of lipid-enriched diets as a treatment for lipid deficiency in the brain

Even though the phenotypes of the GFAP-SCAP and P0-SCAP mutant mice are quite complex and diverse, we found in both cases symptoms that also in humans are found in nervous system diseases that are caused by altered lipid metabolism. PO-SCAP mice, which develop severe congenital hypomyelination, represent a valuable model for peripheral neuropathies that are associated with compromised lipid metabolism, e.g., diabetic peripheral neuropathy and Zellweger syndrome (Horrobin 1997; Chrast et al. 2011). GFAP-SCAP mice display symptoms, such as microcephaly; motor dysfunction and impaired learning that are also found in neurodevelopmental lipid disorders, e.g., NPD-C and SLOS. Moreover, also in the most common central neurodegenerative diseases such as Parkinson and Huntington disease, as well as multiple sclerosis, lipid metabolism is impaired and progressive motor dysfunction is observed. Therefore, we can use these two models to specifically study the consequences of perturbed lipid metabolism in the PNS and CNS and develop treatments that specifically rescue the effect of these perturbations.

In both P0-SCAP and GFAP-SCAP mutant mice, we could show that endogenous lipid synthesis via the SCAP/SREBPs pathway is crucial for proper development and function of the nervous system, but that exogenous lipid uptake may serve as an alternative lipid source. Using the GFAP-SCAP model, we have shown, that a fat-enriched diet is a valuable therapy to treat symptoms associated with defective astrocyte lipid metabolism, including paroxysmal dyskinesia, impaired acoustic startle response, as well as premature death. In PO-SCAP mutants we found a rescue of myelin by exogenous lipid uptake, and we showed that addition of lipoproteins to mutant myelinating Schwann cells in culture increases myelination. Together, these findings support the development of treatments that use lipid supplementation for diseases that are directly associated with deficits in lipid metabolism.
Dietary lipid treatments have recently emerged as an alternative intervention for a variety of neurological disorders. Next to the diet we used, which is enriched in monounsaturated lipids and cholesterol (Camargo et al. 2012) and the cholesterol enriched diet for treatment of PMD mice (Saher et al. 2012), ketogenic diets, which are high in fat and low in carbohydrates, were shown to be efficient for treatment of refractory epilepsy in patients and mice (Todorova et al. 2000). In transgenic models for Alzheimer disease ketogenic diet decreases beta-amyloid deposition (Van der Auwera et al. 2005) and improves motor deficits in a transgenic model of amyotrophic lateral sclerosis (Zhao et al. 2006). Recently, it was shown that a ketogenic diet ameliorates motor deficits as well as synaptic plasticity, learning and memory, in the murine experimental autoimmune encephalomyelitis model of multiple sclerosis (Kim do Y., 2012).

Taken together, we have shown that deficient endogenous lipid synthesis and perturbed lipid metabolism causes disabling defects in the nervous system. However, we also show that these deficits can be directly treated by lipid supplementation in the diet. The benefits of lipid supplementation that we observed were however partial for most of the phenotypes, which strongly warrants for optimization of diets in terms of their composition and timely application. Importantly, we propose that application of lipid-supplemented diets may form a valuable treatment for neurological disorders that are associated with compromised brain lipid metabolism.
References


