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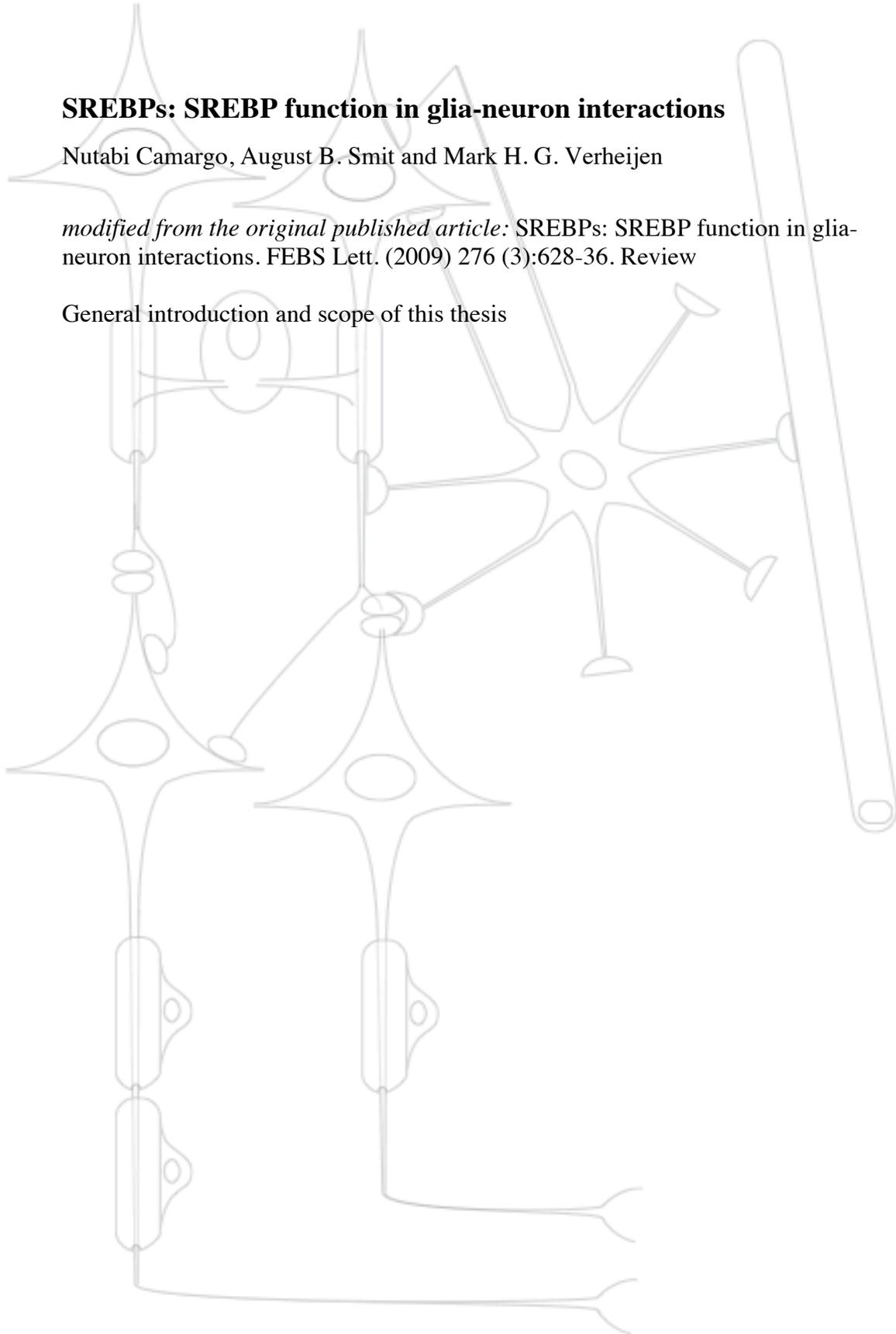
CHAPTER 1

SREBPs: SREBP function in glia-neuron interactions

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General introduction and scope of this thesis



1- Cells of the nervous system

Our understanding of the architecture and function of the nervous system started to see the light at the end of the 19th century. Even though Virchow reported the existence of neuronal and non-neuronal elements in the brain in 1846 (Virchow 1846), only the development of elaborated staining techniques by Camillo Golgi in 1890 allowed the visualization of various principal nervous system elements, which appeared to have sophisticated morphologies and high levels of organization. Using Golgi's metal impregnation system, Santiago Ramon y Cajal identified 3 main cell types in the brain in 1913 (Ramon y Cajal 1913): neurons (the first element), astrocytes (the second element) and a remaining group of small, round nuclei cells (the third element). Subsequent elucidations by Del Rio Hortega on the third element (Rio Hortega 1928) led to further distinctions of two main categories, oligodendrocytes, which turned out to be more closely related to astrocytes and therefore belong to the second element, and a rather different cell type, which originated outside of the nervous system and is now known as microglia (Rio Hortega 1921). Functional studies of these cellular elements became possible after the development of cell electrophysiology and resulted in the definition of two categories of cells: neurons and glia, that appeared to differ in their connective properties. Neurons were defined as electrically excitable and connected to each other through synapses and (as we know now) have the ability of sensing stimuli and create, analyse, integrate and transfer information. Glial cells, which didn't respond to electrical stimulation, were thought to be supportive in function allowing neurons to stick together (Glia= glue in greek). This caused a rapid shift in the focus of researchers towards the function of neurons, leaving glial cells aside. The discovery of immunohistological techniques in 1942 (Coons 1951; Coons 1971) and its application to nervous tissues provided a next step in the elucidation of the origin, development and localization of specific cell types in the brain. Importantly, it revealed the implication of glial cells in pathological situations, which made researchers realize that most neurological dysfunctions were accompanied by changes in glial cell morphology and immunoreactivity. However, only since advancements in imaging techniques, in particular, the application of fluorophore indicators to visualize intracellular ion changes in living cells, it became possible to

investigate the physiology of glia which resulted in the discovery that glial cells, not being electrically excitable, do display calcium excitability (Barres, Chun et al. 1989). Since then, increasing attention has been paid to glial cells as they have been recognized to play an important role in brain physiology in pathological situations. To date it is obsolete to try to understand the function of neurons without taking in account their intimate relationship with glial cells.

Glial cells are currently divided in two main groups, microglia and macroglia. Microglia are the macrophages of the nervous system, they originate from the peripheral mesodermal tissue, and belong to the hematopoietic system. They have phagocytic actions and as such have been called the immune cells of the nervous system. Since microglia are not in the focus of this thesis I refer for further details on microglia to the review of (Ransohoff and Cardona 2010). Macroglia can be further subdivided into two main subtypes: 1) myelinating cells; Schwann cells in the peripheral nervous system and oligodendrocytes in the central nervous system and 2) astrocytes (Figure 1).

1.1) Myelinating cells

The rapid saltatory conduction of neural action potentials is crucially dependent on the compact insulating myelin layers around axons. The myelin membrane is an organelle synthesized by Schwann cells in the PNS, and by oligodendrocytes in the CNS.

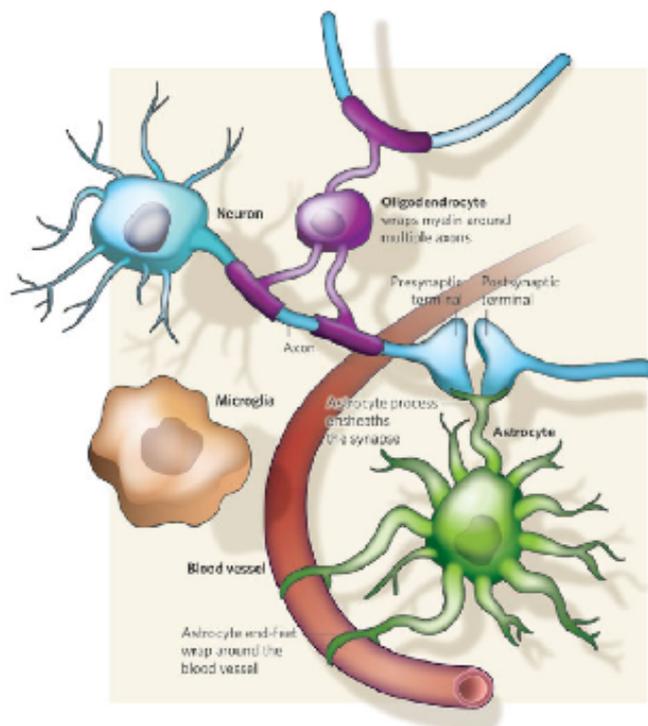


Figure 1. Glia-neuron interactions in the central nervous system. Reused from Allen N. and Barres B., 2009, with permission.

In addition to their role as insulators, it has recently been shown that oligodendrocytes and Schwann cells maintain the functional integrity of axons (Kassmann and Nave 2008; Nave and Trapp 2008; Quintes, Goebbels et al. 2010). Myelination, in both the PNS and CNS, is a highly regulated process and even though Schwann cells and oligodendrocytes serve similar functions, and the morphology and structure of the myelin made by these two types of cells is alike, there are several differences concerning the signals that drive myelination in the CNS and PNS. Same holds for the origin of the myelinating cells and the configuration of these cells around the axons. Some of these differences are discussed here.

1.1.1) Myelin in the PNS: Schwann cells

During development, myelinating and non-myelinating PNS glial cells generate from neural crest cells. Early delaminating neural crest cells take the ventral migratory pathway and differentiate into neurons and Schwann cell precursors (E12-E13). Schwann cell precursors are defined as Sox10 positive cells, that are tightly associated with neuronal projections at early stages of embryogenesis and are able to migrate long distances along the nerves (Jessen and Mirsky 2005). Once on their destination, Schwann cell precursors differentiate into immature Schwann cells (E13-E15), and are still found in the mouse nerves at later stages of embryonic development and at early post-natal days (E17-P0). The post-natal fate of immature Schwann cells is determined by which axons they randomly associate with, with myelination being selectively activated in those cells that happen to envelop single large diameter axons. Myelination by Schwann cells is a highly dynamic process that parallels CNS gliogenesis, with a peak at 2 weeks after birth in mice and a steady state in the adult nerve. Schwann cells myelinate a small segment of only one axon, and thereby outnumber axons by far. Differentiation of Schwann cells has been shown to be highly dependent on signals from the axon, in particular neuregulins and neurotrophins (Chan, Watkins et al. 2004; Brinkmann, Agarwal et al. 2008). The myelination process results in a fast enrichment of lipids and myelin-specific proteins in the nerve (Verheijen, Chrast et al. 2003), in line with lipids and myelin specific proteins being essential building blocks of the myelin membrane, as will be discussed below.

1.1.2) Myelin in the CNS: oligodendrocytes

Oligodendrocyte precursors originate from neuroepithelial cells in the ventricular zone and in ventral regions of the neural tube in embryonic development. Differentiation and maturation of these precursors into fully myelinating oligodendrocytes occurs between P5 and P21, with a peak at P14 (for a complete review on oligodendrocyte biology see (Baumann and Pham-Dinh 2001)). In contrast to Schwann cells, oligodendrocytes extend several processes that make small segments along the same and/or different axons, as such oligodendrocytes myelinate multiple axons. Whereas neuronal derived neuregulin 1 type 3 has been shown to play a pivotal role in PNS myelination (Michailov, Sereda et al. 2004), the regulation of oligodendrocyte differentiation is not dependent on this signalling pathway (Brinkmann, Agarwal et al. 2008). As in the PNS, myelination in the CNS requires the timely regulated expression of myelin proteins and membrane enrichment in lipids (Saher, Brugger et al. 2005; Saher, Quintes et al. 2011) .

1.2) Astrocytes: the multifunctional cells of the CNS

Astrocytes are the most abundant cells in the brain, they are found throughout the central nervous system, including the white matter. Astrocytes, like neurons and oligodendrocytes, are derived from neuroepithelial cells in the ventricular zone of the CNS at early stages of development (Rao and Mayer-Proschel 1997; Kriegstein and Alvarez-Buylla 2009). Upon differentiation from neuroepithelial cells, the formation of astrocytes is characterized by the expression of the glial fibrillary acidic protein (GFAP), the major intermediate filament in the astrocyte cytoskeleton. Although GFAP is already expressed during embryonic development (~E8), massive GFAP expression takes place in the early postnatal days (P0 to P14), in parallel to neurite outgrowth and synapse formation and the peak of CNS myelination (Sancho-Tello, Valles et al. 1995; Bushong, Martone et al. 2004), indicative of astrocyte proliferation and differentiation concomitant to those processes.

GFAP is expressed by a large number of glial cells in different brain structures. These cells are therefore altogether categorized as astroglia. Some of the main astroglia populations are Bergmann glia in the cerebellum, Muller glia in the retina, tanycytes in the hypothalamus, pituicytes in the pituitary, protoplasmic astrocytes in the cortex and hippocampus, and fibrillary astrocytes in the white matter

(Nolte, Matyash et al. 2001). GFAP is also found in embryonic radial glia, a type of glia that is used as scaffold for migration by neurons during cortical layer formation, but which is only present during a restricted time in embryonic development (E8-E16 in mouse). This role of acting as scaffold is also seen postnatally for mature astroglia, such as Bergmann glia in the cerebellum, where it not only serves for neuronal migration but also directs neurite outgrowth influencing the shape of the Purkinje cell dendritic tree (Lordkipanidze and Dunaevsky 2005). Most of the above-mentioned astrocytes share, besides the expression of GFAP, a wide range of functions. They contact blood capillaries and therefore have a pivotal role in forming the blood-brain barrier, participating in the selective uptake of circulating molecules. Moreover, astrocytes in the grey matter are in close contact with neurons, where they actively participate in neuronal transmission. In the cortex and hippocampus, astrocytes have been shown to occupy a defined territory, called an astrocytic island, probably enabling a high degree of synchronization between neurons that belong to one island (Halassa, Fellin et al. 2007). Finally, astrocytes form functional networks by contacting each other through gap junctions and can display increases in internal calcium levels that propagate from one astrocyte to the next for long distances (Enkvist and McCarthy 1994; Fiacco and McCarthy 2006). These waves have been suggested to be important during early postnatal development (Meier, Kafitz et al. 2008) and shown to modulate neuronal activity (Min and Nevian 2012). Importantly, these waves are generated by synaptic release of neurotransmitter for which astrocytes express many receptors (*e. g.*, glutamate, purines, noradrenaline, acetylcholine receptors) (Porter and McCarthy 1997; Fellin and Carmignoto 2004; Fields and Burnstock 2006). Another important feature shared by astrocytes in the CNS is the expression of apolipoproteins (Pitas, Boyles et al. 1987; Hayashi 2011). This characteristic has placed astrocytes as the hypothetical major suppliers of lipids to neurons and other cells in the brain, as will be discussed below.

1.3) Glia-neuron interactions

The introduction of glia-neuron interactions as a sub-discipline in neuroscience started a couple of decades ago and has gained increasing interest over the last decade due to the development of electrophysiology, imaging techniques and mouse transgenesis, that together made it possible to determine the function of specific genes

in subpopulations of cells in the nervous system (Barres 1989; Barres 1991; Jahromi, Robitaille et al. 1992; Pfrieger and Barres 1996; Araque, Parpura et al. 1999; Haydon 2001). Moreover, the development of elaborated *in vitro* systems, using highly purified cell populations, has strongly contributed to the elucidation of the effects of glia on neuronal development and function (Hatten, Mason et al. 1984; Ullian, Sapperstein et al. 2001; Thomson, McCulloch et al. 2008). The use of astrocyte-neuron co-cultures has led to the identification of several astrocyte-derived signals that induce neuritogenesis and synaptogenesis, at least *in vitro*. For instance, oleic acid and cholesterol are secreted by astrocytes in high quantities and are well known to promote neurite outgrowth and synapse formation, respectively (Mauch, Nagler et al. 2001; Medina and Taberner 2002; Goritz, Mauch et al. 2005). Other astrocyte factors that were shown to influence neuronal development are extracellular matrix molecules (like thrombospondin 4) (Christopherson, Ullian et al. 2005). Also, neurons co-cultured with oligodendrocytes or Schwann cells are used to follow the different steps of myelination *in vitro* (Thomson, McCulloch et al. 2008). Lately, the addition of astrocytes to co-cultures of oligodendrocytes and neurons has led to the new view that astrocytes may enhance myelination, at least *in vitro* (Watkins, Emery et al. 2008). However, little is known about this type of interaction and the elucidation of the molecular pathways that play a role in this remain largely undetermined.

2) The nervous system; an autonomous organ in lipid metabolism

The CNS and PNS need to be highly active in lipid synthesis, as both are shielded from lipids in the circulation by respectively the blood brain barrier (BBB) and the blood nerve barrier (BNB) (Jurevics and Morell 1994). As such, the nervous system may be viewed as largely autonomous in lipid metabolism. This raises the issue of the identity of the cell type(s) and molecular processes involved in lipid synthesis in PNS and CNS. The functionally diverse glia-neuron interactions include both contact-dependent as well as soluble factors, and involve a wide spectrum of molecules, among which lipids. Also recently, the role of lipids in the pathophysiology of several neurological diseases has been demonstrated. Important transcriptional regulators of lipid synthesis are SREBP transcription factors (sterol-regulatory-binding-proteins), which are known to be involved in diseases associated with dysfunction of lipid metabolism in several organs, *e. g.*, liver, kidney and pancreas (Horton, Goldstein et al. 2002; Shimano 2002; Shimano, Amemiya-Kudo et al. 2007). The view starts to

emerge that glia SREBPs are also involved in neurological diseases. Below, I will discuss SREBPs, and the current understanding of the role of SREBP in glia-neuron interactions in health and disease.

2.1) Sterol-regulatory-binding protein (SREBPs)

The SREBPs belong to the family of basic helix-loop-helix-leucine zipper (bHLH-Zip) transcription factors that are known to regulate lipid metabolism in liver and adipose tissue. The SREBP family consists of SREBP-1a, SREBP-1c and SREBP-2 (Horton, Goldstein et al. 2002). SREBP-1c and SREBP-2 preferentially govern the regulation of genes involved in fatty acid and cholesterol metabolism, respectively (Figure 2), whereas SREBP-1a activates both pathways (Horton, Goldstein et al. 2002; Shimano 2002). SREBP-1a is expressed ubiquitously at low levels, in contrast to the differentially regulated expression of SREBP-1c and SREBP-2. Expression of SREBP-2 is induced under conditions of sterol-depletion, whereas SREBP-1c expression is under the control of insulin, glucose and fatty acids in several tissues, among which Schwann cells (Horton, Goldstein et al. 2002; Shimano 2002; de Preux, Goosen et al. 2007). A characteristic of the SREBP transcription factors is their post-translational activation by SCAP, which is under the control of lipid levels (Figure 3). SCAP acts as a sterol sensor, which in sterol-depleted cells escorts the SREBPs from the endoplasmic reticulum (ER) to the Golgi apparatus, where two membrane-associated proteases, S1P and S2P activate them by cleavage. The mature and transcriptionally active forms of the SREBPs translocate to the nucleus where they bind gene promoters containing sterol regulatory elements (SRE). These SREBP target genes are involved in synthesis and metabolism of cholesterol and fatty acids (Horton, Goldstein et al. 2002; Shimano 2002) (Figure 2).

2.2) Role of Schwann cell SREBP in myelination

The electrical insulating property of the myelin membrane is provided by its high and characteristic lipid content. While it has been suggested that many of these myelin lipids are synthesized in the nerve itself, as was demonstrated for cholesterol (Jurevics and Morell 1994; Morell and Jurevics 1996), the factors regulating their synthesis have largely stayed unknown. Verheijen et al. reported expression profiling of the peripheral nerve during myelination, which has provided many insights into the

transcription regulation of myelin lipid metabolism, and suggest a central role for SREBPs (Verheijen, Chrast et al. 2003).

The biochemical characteristic that distinguishes myelin from other plasma membranes is its exceptional high lipid-to-protein ratio. The myelin membrane contains myelin specific proteins, such as Myelin Protein Zero (MPZ), PMP22, MAG and MBP, however, no myelin specific lipids. However, whereas all major lipid classes are present in myelin, as in other membranes, the myelin membrane is enriched in galactosphingolipids, saturated long-chain fatty acids and cholesterol, the

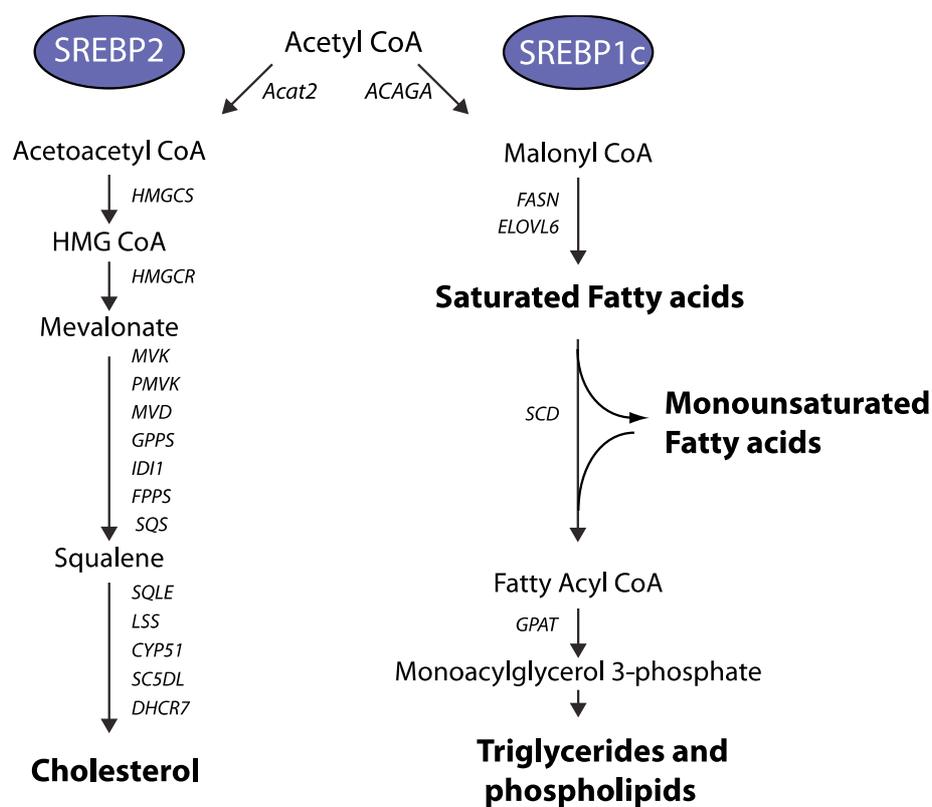


Figure 2. Genes regulated by SREBPs for the synthesis of cholesterol, fatty acids and triacylglycerides. SREBP1c preferentially governs the transcriptional activation of genes involved in fatty acid and triglycerides metabolism, SREBP2 in cholesterol metabolism, whereas SREBP1a (not shown in the figure) activates both pathways. CYP51, lanosterol 14 α -demethylase; DHCR, 7-dehydrocholesterol reductase; ELOVL6, elongation of long chain fatty acids family member 6; FASN, fatty acid synthase; FDPS, farnesyl diphosphate synthase; GGPS, geranylgeranyl pyrophosphate synthase; GPAT, glycerol-3-phosphate acyltransferase; HMGCR, hydroxymethylglutaryl-Coenzyme A reductase; HMGCS, HMG CoA synthase; DDI, isopentenyl-diphosphate delta isomerase; LSS, lanosterol synthase; MVK, mevalonate kinase; MVD, mevalonate diphosphate decarboxylase; PMVK, phosphomevalonate kinase; SC5DL, sterol-C5-desaturase; SCD, stearyl CoA desaturase; SQS, squalene synthase; SQLE, squalene epoxydase.

later being the most abundant lipid (see (Garbay, Heape et al. 2000) for a comprehensive review on the molecular constituents of PNS myelin).

2.2.1) SREBP and myelin cholesterol synthesis

With the membrane surface area expanding a spectacular 6500-fold during myelination (Webster 1971), it is of interest that almost all of the cholesterol in the myelin membrane is synthesized by the nerve itself (Jurevics and Morell 1994). In line with this, myelination and remyelination is not affected by deletion of the LDL receptor (Goodrum, Fowler et al. 2000).

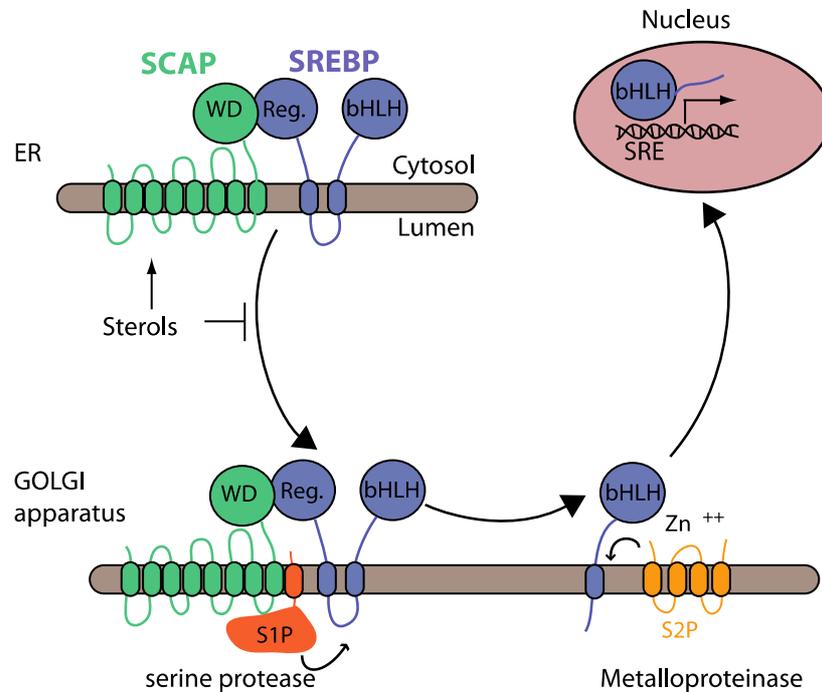


Figure 3. Model for the sterol-mediated proteolytic release of SREBPs from membranes. SREBPs belong to the family of basic helix-loop-helix-leucine zipper (bHLH-Zip) transcription factors and regulate lipid metabolism. SREBP transcription factors crucially rely on post-translational activation involving the sterol sensor SCAP. When sterol levels are low, SCAP escorts the SREBPs from the ER to the Golgi, where they are activated through sequential processing by the membrane-associated proteases, S1P and S2P. The resulting mature and transcriptionally active forms of the SREBPs (bHLH-Zip domain) translocate to the nucleus where they bind genes containing sterol regulatory elements. Adapted from Horton, J. D et al. 2002.

Longstanding studies on cholesterol biosynthesis for the myelin membrane showed that exposure of rats to a diet containing tellurium, which blocks the conversion of squalene epoxydase, leads to an accumulation of squalene and an absence of cholesterol in the nerve (Harry, Goodrum et al. 1989). This results in rapid PNS demyelination for a week, after which remyelination occurs, even with continuing tellurium exposure (Wiley-Livingston and Ellisman 1982). Together, these studies show that glial cholesterol synthesis is crucial for myelin membrane formation and integrity. Observations on the transcriptional control of the cholesterol pathway are in line with this since this process follows the active period of myelination (Fu, Goodrum et al. 1998; Nagarajan, Le et al. 2002; Verheijen, Chrast et al. 2003). Importantly, SREBP-2 follows the same time course of expression (Salles, Sargueil et al. 2003; Verheijen, Chrast et al. 2003; de Preux, Goosen et al. 2007). Together with the demonstrated role for SREBP-2 in cholesterol metabolism in other tissues, this points to an important role for Schwann cell SREBP-2 in the synthesis of myelin cholesterol (Figure 4). It should be noted that expression levels of SREBP-1a in Schwann cells are continuously very low, while SREBP-1c expression is strongly upregulated after myelination in adults (Verheijen, Chrast et al. 2003; Leblanc, Srinivasan et al. 2005; de Preux, Goosen et al. 2007), as will be discussed below.

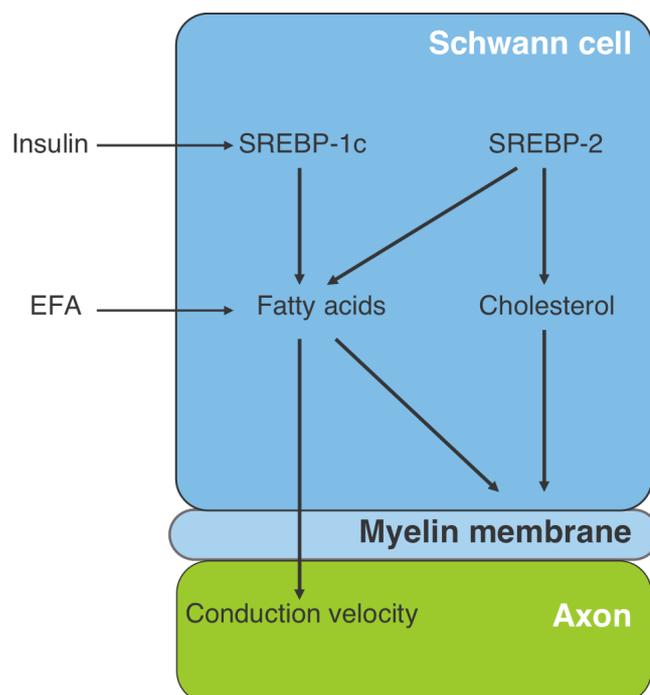


Figure 4. Schematic diagram on the role of Schwann cell SREBPs in myelination. SREBP-2 predominantly regulates the expression of enzymes involved in cholesterol synthesis, and to a lesser extent fatty acid and phospholipid metabolism, necessary for the myelin membrane. SREBP-1c is in adults under the control of insulin, and is predominantly involved in myelin fatty acid and phospholipid metabolism and possibly in direct effects of fatty acids on functioning of the axon. EFA: essential fatty acids.

Interestingly, expression analysis of SREBPs in two different mouse models for PNS dysmyelination, the Trembler mouse (Salles, Sargueil et al. 2003) and the Krox-20 knockout mouse (Leblanc, Srinivasan et al. 2005), shows reduced expression of SREBP-2 but not of SREBP-1a or -1c. Together with the observations that dysmyelination in these models is accompanied by reduced myelin lipid synthesis (Garbay, Heape et al. 2000; Leblanc, Srinivasan et al. 2005), these data support a major role for SREBP2 in myelin lipid synthesis. It should be noted that ectopic expression of Krox-20 in Schwann cells *in vitro* induces expression of lipogenic genes (Nagarajan, Svaren et al. 2001). Also, other *in vitro* studies suggest that while Krox-20 does not induce the expression of SREBP-2, it acts with SREBPs on the activation of lipogenic gene promoters, such as HMGcR and SCD-2 (Leblanc, Srinivasan et al. 2005).

In summary, both expression analysis and molecular approaches *in vitro* indicate a role for SREBP-2 in the control of the cholesterol synthesis pathway during myelination.

2.2.2) *SREBP and fatty acyl components of myelin lipids*

Myelin membrane lipids have a fatty acid composition that is distinguishable from other membranes; they have high levels of oleic acid [C18:1 (n-9)], which is the major myelin fatty acid, and of very long-chain saturated fatty acids (>C18) (Garbay, Heape et al. 2000). Interestingly, the ratio between C18:1 and C18:2 increases strongly during myelination (Garbay, Boiron-Sargueil et al. 1998). In line with these observations stearoyl-CoA desaturase 2 (SCD2), which may desaturate C18:0 into C18:1, follows the same time course of expression as structural myelin protein genes (Garbay, Boiron-Sargueil et al. 1998; Salles, Sargueil et al. 2002; Verheijen, Chrast et al. 2003). The observations that SREBP-1 and SREBP-2, as well as their target genes fatty acid synthase (FASn) and SCD2, are upregulated in the developing peripheral nerve (Salles, Sargueil et al. 2002; Verheijen, Chrast et al. 2003; de Preux, Goosen et al. 2007) suggest an important role for SREBPs in myelin fatty acid composition, and as such in fatty acyl components for membrane phospholipids.

Unlike the expression of SREBP-2 and cholesterologenic enzymes, that are downregulated after the active myelination period, the expression of Schwann cell SREBP-1c is strongly upregulated in the mature nerve (Verheijen, Chrast et al. 2003;

de Preux, Goosen et al. 2007). This suggests that the mature nerve is highly active in fatty acid metabolism. In line with this is our observation that adult peripheral nerves contain high amounts of storage lipids in their epineurial compartment, and that local lipid metabolism is important for normal nerve function (Verheijen, Chrast et al. 2003). This aligns furthermore with a number of human diseases that produce peripheral neuropathies and are associated with altered lipid metabolism. Refsum's disease is caused by defective Schwann cell branched chain fatty acid oxidation and leads to a sensory-motor demyelinating neuropathy (Jansen, Ofman et al. 1997). Also, mutation of *Lpin1*, an phosphatidic acid phosphatase that serves as a key enzyme in the biosynthetic pathway of triglycerides and phospholipids, causes lipodystrophy that includes the epineurial compartment, and is associated with demyelinating peripheral neuropathy (Verheijen, Chrast et al. 2003). Recent observations on a Schwann cell specific *Lpin1* mutant mouse suggest that depletion of *Lpin1* function in Schwann cells only is sufficient to induce a demyelinating phenotype (Nadra, de Preux Charles et al. 2008). Whether lipids from the epineurial compartment are implicated in functioning of axons and Schwann cells in the endoneurial compartment is an intriguing hypothesis that remains to be evaluated.

The observation of de Preux et al., that SREBP-1c is expressed in Schwann cells of adult peripheral nerve, together with observations of others that the action of SREBP-1c in multiple tissues is affected in diabetes, suggest that malfunction of SREBP-1c may underlie the pathological changes associated with diabetic peripheral neuropathy (DPN) (Sima 2003; de Preux, Goosen et al. 2007). Type 1 diabetes mellitus is thought to impair polyunsaturated fatty acid (PUFA) metabolism by decreasing fatty acid desaturase activity, resulting in lower PUFA content in membrane phospholipids of multiple tissues, including the peripheral nerve (Horrobin 1997). Dietary supply of PUFAs improved the impaired NCV in a rodent type I DPN but also in humans (Horrobin 1997). In line with these observations, PUFAs have been demonstrated to modify the activity of axonal Na⁺/K⁺-ATPases (Vreugdenhil, Bruehl et al. 1996). Interestingly, SREBP-1c has been demonstrated to mediate the insulin-induced transcription of the stearoyl-coA desaturase (SCD1), delta-5 desaturase (D5D) and delta-6 desaturase (D6D) (Nakamura and Nara 2002). While SCD1 is involved in the biosynthesis of monounsaturated fatty acids (MUFAs), such as oleic acid, a major constituent of the myelin membrane, D5D and D6D are required

for the metabolic conversion of gamma-linolenic acid into PUFAs and implicated in reduced NCV of diabetic patients. In line with this, de Preux and co-workers, recently reported that expression of SREBP-1c and its target genes FAS and SCD-1 are downregulated in Schwann cells in rodent models of type-1 diabetes (de Preux, Goosen et al. 2007). They also showed that fasting and refeeding of rodents strongly affected expression of the SREBP-1c pathway. In line with this, insulin affected SREBP-1c expression in Schwann cells by activation of the SREBP-1c promoter (Figure 4). Clearly, the expression of Schwann cell SREBP-1c is affected by diabetes and nutritional status, indicating that disturbed SREBP-1c regulated lipid metabolism may contribute to the pathophysiology of DPN.

Taken together, published studies indicate that fatty acid and phospholipid synthesis necessary for the formation of the myelin membrane may be regulated by both Schwann cell SREBP-1c as well as SREBP-2. Interestingly, SREBP-1c may as well be important for functioning of the adult peripheral nerve.

2.2.3) Schwann cell SREBPs: conclusion and perspective

The temporal expression profile of the SREBPs during myelination follows the expression of lipogenic enzymes, and is thereby in keeping with a role for SREBPs in synthesis and metabolism of cholesterol and fatty acids for the myelin membrane. In analogy with the demonstrated role of the different SREBP isoforms in liver (Horton, Goldstein et al. 2002; Shimano, Amemiya-Kudo et al. 2007), the action of SREBP-2 in Schwann cells may predominantly be the transcriptional regulation of cholesterol synthesis, whereas Schwann cell SREBP-1c may function, possibly in concert with SREBP-2, in synthesis and metabolism of fatty acids and phospholipids (Figure 4). Whether myelination is indeed dependent on the action of SREBPs in Schwann cells remained to be determined and is part of the research described in this thesis (chapter 2). In addition, the elevation of SREBP-1c expression in the adult peripheral nerve suggests an active role for Schwann cell SREBP-1c in functioning of the nerve, a role that may be compromised in the pathophysiology of diabetic peripheral neuropathy. The factors regulating SREBP activity in Schwann cells are so far unclear. Post-translational activation of SREBPs in liver is induced by cholesterol depletion. Whether the activation of SREBP is also regulated by sterols in Schwann cells is so far unclear, but would be in line with the suggestion that synthesis of cholesterol-rich

myelin membrane may lead to transient cytosolic cholesterol depletion (Fu, Goodrum et al. 1998).

Studies on the transcriptional control of myelin lipid metabolism have all focused so far on Schwann cells, and the expression of SREBP in oligodendrocytes have not yet been reported. Oligodendrocytes are highly active in lipid metabolism, and have been demonstrated to synthesize cholesterol for the myelin membrane themselves (Saher, Brugger et al. 2005). This suggests that the observed roles of SREBPs in Schwann cells may also have their counterparts in CNS myelination by oligodendrocytes, although this remains to be proven.

2.3) Brain lipid metabolism: implication of astrocyte SREBP in neuronal function

The brain is remarkably different in its lipid composition than other organs. It is highly enriched in poly-unsaturated fatty acids (PUFA) and cholesterol. Accordingly, the brain contains about one quarter of the total amount of cholesterol in the body although it comprises only 2% of the total body weight (Dietschy and Turley 2004). This raises the questions whether there are specific functions for lipids in the brain and which celltype(s) are involved in their synthesis.

Brain lipids have been attributed a wide spectrum of relevant physiological functions. For instance, lipids may function as building blocks for membranes and are therefore important in myelination (Garbay, Heape et al. 2000), neurite outgrowth (Vance, Hayashi et al. 2005), and synaptogenesis (Slezak and Pfrieder 2003). In addition, lipids may act as signalling molecules in brain communication (Bazan 2003). As such, lipid homeostasis in the nervous system is an important process that requires a high level of regulation. Importantly, many studies have demonstrated that the cells playing a central role in the synthesis and metabolism of lipids in the brain are not neurons but glial cells. While the oligodendrocytes synthesize lipids as constituents of myelin, as has been discussed above, astrocytes on the other hand have been proposed to supply lipids to neurons and thereby regulate neurite outgrowth and synaptogenesis (Slezak and Pfrieder 2003). Astrocytes are the most abundant cells in the brain, and thought to have multiple functions. They participate in uptake of nutrients from the blood brain barrier by surrounding the capillary with their endfeet (Haydon and Carmignoto 2006). On the other end, astrocytes are closely associated with the pre-synaptic and post-synaptic terminals and as such are part of the so-called

tripartite synapse (Haydon and Carmignoto 2006; Halassa, Fellin et al. 2007). It has been estimated that one astrocyte can contact 300 to 600 neuronal synapses, which led to the proposal that astrocytes are able to synchronize a group of synapses (Halassa, Fellin et al. 2007). By being in contact with capillary as well as with multiple synapses, astrocytes may supply neurons with nutrients in accordance to the intensity of their synaptic activity. In addition, they may act to affect synaptic function over a long range by astrocyte-astrocyte coupling. In the mammalian brain, astrocyte differentiation takes place in the early post-natal days when massive synaptogenesis in the CNS occurs. In line with this, many studies propose that glia support neuronal survival, enhance neurite outgrowth and increases synaptogenesis. Intriguingly, recent insights indicate that astrocytes may not only do this via direct contact (Hama, Hara et al. 2004), but also via secreted factors, which includes fatty acids and cholesterol.

2.3.1) Involvement of astrocyte SREBP in fatty acid synthesis: regulation of neurite outgrowth and synaptic transmission

In a series of studies, Taberero and Medina have demonstrated that astrocytes synthesize and release oleic acid, which on its turn induces differentiation of cocultured neurons. Oleic acid was shown to be enriched in membrane phospholipids in neuronal growth cones, but was also shown to stimulate neuronal differentiation (Rodriguez-Rodriguez, Taberero et al. 2004). The synthesis of oleic acid by astrocytes was demonstrated triggered by the transit of albumin, a fatty acid binding protein present in the developing brain, into the astrocyte endoplasmatic reticulum compartment. This transit of albumin correlated with induction of SREBP-1 activation and subsequent upregulation of SCD-1, an enzyme involved in oleic acid synthesis, in astrocytes but not neurons (Taberero, Granda et al. 2002). In line with this, SREBP-1 has been detected in several regions of the rodent brain at different ages (Okamoto, Kakuma et al. 2006). Together this indicates a role for astrocyte SREBP-1 in the synthesis of mono-unsaturated fatty acids (MUFAs) and the subsequent differentiation of neighbouring neurons (Figure 5).

Importantly, besides MUFA, also PUFAs have been demonstrated to strongly stimulate neurite outgrowth (Darios and Davletov 2006). In addition, PUFAs have been demonstrated to function in synaptic transmission. For instance, docosahexaenoic acid (Trajkovic, Dhaunchak et al. 2006), was demonstrated to

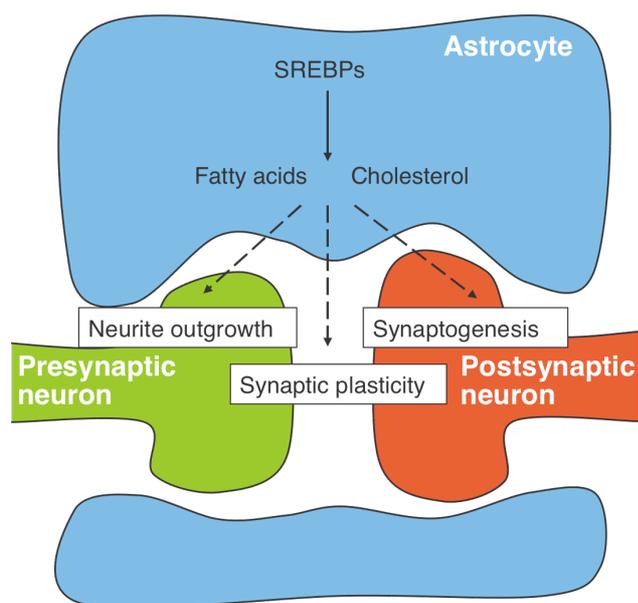
modulate ion currents in isolated hippocampal neurons (Vreugdenhil, Bruehl et al. 1996). Also, arachidonic acid (AA) was reported to stimulate neurotransmitter release via direct binding to syntaxin, a component of the synaptic vesicle release machinery (Connell, Darios et al. 2007). Interestingly, *C. elegans* lacking D6D, a desaturase essential for long chain PUFA synthesis, were found to be defective in neurotransmission, probably by a lack of synaptic vesicle formation (Lesa, Palfreyman et al. 2003). Whereas large amounts of PUFA, predominantly DHA and AA, are found in the brain, the origin of these is unclear. Multiple sources for PUFA in the brain have been described among which uptake of PUFA from the circulation, either directly through the diet or via transformation by the liver, and via local synthesis of PUFA in glial cells (Green and Yavin 1993). The developing brain was found to make its own PUFA from essential fatty acids (EFA) and to incorporate these PUFA into phospholipids (Green and Yavin 1993). Interestingly, Moore and co-workers, demonstrated that astrocytes, unlike neurons, are active in desaturation and elongation of EFAs into PUFAs (Moore 2001). In fact, neurons of different brain regions were found to take up astrocyte-derived PUFA and to subsequently incorporate them into phospholipids. In line with this, the desaturases D5D and D6D, were found expressed in astrocytes (Innis and Dyer 2002). By analogy with the role of SREBP-1 in the regulation of D5D and D6D expression in liver (Matsuzaka, Shimano et al. 2002), astrocyte SREBP-1 might be involved in synthesis of PUFAs and as such might play an active role in synaptic communication. Whether neuronal activity on its turn is able to regulate SREBP activity in astrocytes is an intriguing possibility that remains to be determined. In this respect it should be noted that the regulation of SREBP-1 expression and activity in the brain differs from that in the periphery. Nutritional status and insulin levels are known to regulate SREBP-1 expression in Schwann cells in the PNS, as discussed above (de Preux, Goosen et al. 2007), however, not in the brain (Okamoto, Kakuma et al. 2006). Interestingly, the expression of SREBP-1 in the brain increases in mice during aging (Okamoto, Kakuma et al. 2006), a phenomenon also observed in the peripheral nerve (de Preux, Goosen et al. 2007). The meaning of this aging-related increase in SREBP-1 in both the PNS and CNS is at this moment unclear, but may indicate an elevated need for local fatty acid metabolism. Taken together, SREBP-1 plays an important role in synthesis of mono-and poly-unsaturated fatty acids in astrocytes, and as such in glia-

neuron interactions that involve fatty acids, such as neurite outgrowth and synaptic transmission (Figure 5).

2.3.2) Involvement of astrocyte SREBP in cholesterol synthesis: regulation of synaptogenesis and synaptic function

With the CNS being highly enriched in cholesterol, it is remarkable that there is almost no transfer of cholesterol-containing lipoproteins from the plasma to the CNS both in adults as well as during postnatal development (Dietschy and Turley 2004). Analysis of cholesterol synthesis using radioactive labelling techniques has shown that almost all of the cholesterol in the CNS is synthesized in situ (Turley, Burns et al. 1996). Accordingly, brain expression of SREBP-2 and several target genes involved in cholesterol synthesis have been reported (Tarr and Edwards 2008). Astrocytes have been demonstrated to express SREBP2, which is activated during lipoprotein assembly (Ito, Nagayasu et al. 2002). In line with this, astrocytes are the main ApoE-producing cells in the CNS (Boyles, Pitas et al. 1985), while neurons abundantly express ApoE-receptors (Zhuo, Holtzman et al. 2000). In addition, transgenic mice lacking neuronal synthesis of cholesterol, by conditional inactivation of the squalene synthase in cerebellar neurons, did not show differences in brain morphology nor in behavior (Funfschilling, Saher et al. 2007). Clearly, transfer of lipids from glia to neurons plays an important role in neuronal lipid homeostasis.

Figure 5. Schematic of the proposed roles of astrocyte SREBPs in the tripartite synapse. Astrocyte SREBPs regulates the synthesis of monounsaturated and polyunsaturated fatty acids and cholesterol that after secretion are bound by neuronal structures and affect neurite outgrowth, synaptogenesis and synaptic plasticity.



Most synapses in the developing brain are formed after the differentiation of astrocytes (Pfrieger and Barres 1996; Ullian, Sapperstein et al. 2001), and it was demonstrated that astrocytes are required for the formation, maturation and maintenance of synapses in neuronal cultures (Ullian, Sapperstein et al. 2001; Slezak and Pfrieger 2003). The synapse-promoting signal released by astrocytes in these cultures was, surprisingly, demonstrated to be cholesterol complexed to apolipoprotein E (apoE)-containing lipoproteins (Mauch, Nagler et al. 2001). Cholesterol is a major component of neuronal membranes and is a component of specialized microdomains, called lipid rafts, which are required presynaptically for the formation of synaptic vesicles (Thiele, Hannah et al. 2000) and postsynaptically for the clustering and stability of receptors (Allen, Halverson-Tamboli et al. 2007). These findings argue for a prominent role of SREBP-2 and astrocyte-derived cholesterol in synaptic development and function. In addition, it may be speculated that via similar mechanisms astrocytes potentially regulate synaptic plasticity in the adult brain. In line with this, the ApoE receptor LRP (LDL-receptor related protein) has been shown to play an active role in synaptic plasticity in the mouse hippocampus (Zhuo, Holtzman et al. 2000), whereas pharmacological inhibition of cholesterol synthesis inhibits synaptic plasticity in rat hippocampal slices (Matthies, Schulz et al. 1997). Finally, treatment of human astrocytoma cells lines with anti-psychotic and anti-depressants drugs induced activation of SREBPs and subsequent cholesterol synthesis, whereas these drugs had only little effect on the SREBP pathway in human neuronal cell lines, suggesting that the action of such drugs on synaptic transmission may be primary on astrocytes (Ferno, Skrede et al. 2006). Taken together, this implies that SREBP in astrocytes may function in the controlled supply of cholesterol to synaptic structures, and thereby contributes in the formation and behavior of lipid rafts and as such in synaptic function.

2.3.3) A proposed role for astrocyte SREBP in neuronal function

The relative autonomy of the CNS in metabolism of cholesterol and fatty acids, together with the importance of these lipids for neuronal development and synaptic functioning, requires a high activity of lipid synthesis in the brain. Analogous to the liver where SREBP activity is involved in lipid synthesis for supply to the periphery, we propose that SREBP in astrocytes is involved in lipid synthesis for supply to

neurons (Figure 5). Whether neurons are indeed dependent on astrocyte-derived lipids, and as such rely on the action of SREBPs in astrocytes, is one of the main subjects of this thesis and has been approached experimentally in chapters 3 and 4.

Notably, a high number of brain diseases are associated with lipid metabolism dysfunction. For instance, Niemann-Pick disease type C, which causes cognitive deficits and motor impairment in young children has been linked to defective cholesterol transport in astrocytes (Patel, Suresh et al. 1999). In addition, recent studies show a strong connection between lipid metabolism, apolipoprotein E and the neurodegenerative loss of synaptic plasticity in Alzheimer's disease (Poirier 2003). The lipids shown to be involved include cholesterol (Poirier 2003), as well as PUFAs (Calon, Lim et al. 2004). Intriguingly, it was found that the risk of Alzheimer's disease is lower in humans carrying a specific polymorphism in SREBP-1a (Spell, Kolsch et al. 2004). Finally, for Huntington's disease it was demonstrated that expression of the mutant Huntington protein in astrocytes contributes to neuronal damage (Shin, Fang et al. 2005), whereas others demonstrated that this Huntington protein leads to reduced SREBP maturation and consequent reduced cholesterol synthesis (Valenza, Rigamonti et al. 2005). Taken together, these studies are in line with a potential role of astrocyte-derived lipids in the formation, maturation and functioning of synapses, both in health and in disease.

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3) Scope and aim of this thesis

Glia-neuron interactions play essential roles in development and function of the nervous system and are intimately involved in brain pathogenesis. Lipids are major components of the nervous system and perturbed lipid metabolism is observed in a wide spectrum of brain disorders, ranging from brain malformations to neurodegenerative diseases, as well as nervous system repair after injury and neuroprotection. Contrary to neurons, glial cells are well known to be active in lipid synthesis but the exact molecular mechanisms involved in lipid synthesis in the nervous system and the direct consequences of lipid synthesis obliteration in glial cells remained elusive. Based on the above discussed literature, SREBPs seem to play an important role in lipid metabolism of glia of both the PNS and the CNS, and act in diverse processes of glia-neuron interaction, such as myelination, neuronal development, neurite outgrowth, synaptogenesis and synaptic transmission. Accordingly, glia SREBPs may function as a control point of neuronal function. What remains to be determined is the contribution of individual cell types to the lipids found in both the peripheral and central nervous system and the involvement of SCAP and SREBPs in these processes.

This thesis aims to investigate the involvement of SREBP-mediated lipid synthesis in glial cells, with a special focus on the role of SCAP in the regulation of myelination by Schwann cells in the PNS (chapter 2) and the role of SCAP expression in astrocytes as lipid suppliers to the CNS (chapters 3-5). In the first experimental chapter (chapter 2) we made use of a transgenic line to assess the role of Schwann cell SREBP in myelination of the PNS. In the subsequent chapters (chapters 3, 4 and 5) we studied the involvement of astrocyte-SREBP function in lipid metabolism of the central nervous system. For this aim, we made use of a transgenic mouse line generated by cre-mediated recombination of SCAP in GFAP expressing cells. In chapter 3, we extensively characterize the effect of SCAP deletion on lipid composition of the brain and its consequences on survival and behavior of these mice. In addition, we aimed to rescue the mutant phenotypes by feeding the animals with a diet containing high amounts of cholesterol and unsaturated fatty acids, which were specifically decreased in the mutant brains. In chapter 4, we investigated myelin abnormalities in these mice, and aimed to rescue myelin phenotypes by the high fat diet. In chapter 5, we assessed the involvement of deficient lipid synthesis by GFAP

expressing cells in hippocampal function, concerning mainly contextual memory formation and long-term potentiation.

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