An inducible mouse model for postnatal inactivation of astrocyte lipid metabolism

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ABSTRACT

In the mammalian brain, lipids are abundantly present in neuronal membranes and are essential for the formation and development of dendrites, axons and synapses. Neurons have low capacity to synthesize lipids and critically rely on the supply of lipids by astrocytes. We previously showed that SREBP cleavage-activating protein (SCAP) is essential for fatty acid and cholesterol metabolism in astrocytes and that deleting SCAP during embryonic development results in neurological deficits, such as motor deficits, reduced anxiety and impaired presynaptic terminal development. In this study, we generated and analyzed a mouse model that expresses the tamoxifen-inducible CreERT2 under transcriptional control of the astrocyte-specific Glast promoter, in order to investigate the effects of postnatal astrocyte SCAP mutation. First, tamoxifen-induced recombination was observed in cells outside the CNS, in particular in peripheral organs such as the salivary glands and pancreas, particularly when Cre activity was induced during early postnatal development (P1-2), and to a much lesser extent at later developmental time points (P15-17 or P46-48). When SCAP mutation was induced at P15-P17, coinciding with the peak of myelination and after the peak of synaptogenesis, this strongly inhibited lipogenic gene expression in the CNS, specifically in astrocytes, and resulted in impaired myelination and changes in synapse ultrastructure in adults. In particular, SCAP mutants had persistent hypomyelination and reduced size of the active zone and postsynaptic density. These findings suggest that the tamoxifen-inducible CreERT2 inducible mouse model is a powerful tool to dissect the role of astrocyte lipid metabolism during different developmental stages. We found that targeting of cells other than astrocytes should be taken into account when Cre activity is induced during early postnatal development. This study may have important implications for the investigation and understanding of neurological disorders with a postnatal onset of compromised lipid metabolism, such as in Alzheimer’s disease and Huntington’s disease.
INTRODUCTION

In the mammalian central nervous system (CNS), lipid uptake from the circulation is prevented by the blood-brain barrier. Nonetheless, lipids are highly abundant in neuronal membranes. In the CNS, astrocytes are important for de novo lipid synthesis and provide lipids to neurons for proper neurite outgrowth, synaptogenesis and myelination [25,29,163,197]. Synaptic membranes and myelin sheaths, both highly specialized plasma membranes, have unique lipid compositions. Myelin sheaths, consisting of high levels of cholesterol, glycosphingolipids and saturated long-chain fatty acids [179], are wrapped around axons, thereby increasing axonal resistance and allowing fast conduction of action potentials between nodes of Ranvier [198]. Synaptic membranes are enriched in cholesterol and polyunsaturated fatty acids [59,60] and play key roles in synaptic neurotransmission: 1) as important regulators of membrane remodeling during neurotransmitter release from synaptic vesicles, 2) as component of specialized lipid rafts that are localized pre- and postsynaptically, which are required for synaptic vesicle formation and the clustering of neurotransmitter receptors, respectively [43,49]. Accordingly, many neurological disorders in which lipid metabolism is compromised are characterized by myelin and synaptic defects, such as Smith-Lemli-Opitz syndrome and Niemann-Pick disease type C [120,199–201].

We previously demonstrated that cholesterol and fatty acid synthesis in astrocytes relies on sterol regulatory element binding protein (SREBP) transcription factors [56]. SREBPs mediate the transcriptional activation of genes involved in fatty acid and cholesterol metabolism, and are post-translationally activated by the sterol sensor SCAP [50,124]. We recently reported that GFAP-SCAP mice, in which astrocyte SCAP-mediated lipid synthesis is inactivated during the embryonic stage, had impaired presynaptic terminal development, which was accompanied by neurological deficits, including motor deficits and reduced anxiety [56,163]. In the current study, we generated and analyzed a mouse model to induce astrocyte SCAP deletion by a tamoxifen-inducible form of Cre recombinase, allowing the deletion of SCAP at different time points, including during postnatal development and young adults. We used this model to determine the role of astrocyte lipid metabolism in myelination and synapse ultrastructure in adults, when astrocyte-restricted SCAP-SREBP-mediated lipid biogenesis was postnatally inactivated at P1-P2 (before synaptogenesis and myelination), at P15-P17 (after the peak of synaptogenesis and at the peak of myelination) and in young adults at P46-P48 (during synapse and myelin maintenance). We found that tamoxifen-induced recombination was not only restricted to the CNS when induced during early postnatal development (P1-P2); also peripheral organs such as the salivary glands and pancreas were targeted. Peripheral recombination, however, was strongly reduced when induced at later developmental time points. It was found that tamoxifen-induced SCAP mutation at P15-17 resulted in impaired lipid synthesis in corpus callosum and the
hippocampus specifically in astrocytes. This led to persistent hypomyelination and small changes in synapse ultrastructure.

MATERIALS AND METHODS

Mice

All experimental procedures were approved by the local animal research committee (Dierexperimentencommissie VU University) and complied with the European Council Directive (86/609/EEC). SCAP-floxed mice were obtained from the Jackson Laboratory and have been described previously [50]. Glast-CreERT2 mice [202] and Rosa26-tdTomato mice [203] have been described, and were crossed with the SCAP-floxed mice to generate Glast-CreERT2-tdTomato-SCAP mice (referred to as 'Glast-SCAP mice'). Mouse lines were maintained on a C57Bl/6 background. Food (Harlan Teklad, Madison, WI, USA) and water were provided ad libitum. Housing was controlled for temperature, humidity and light-dark cycle (7 AM lights on, 7 PM lights off).

Tamoxifen treatment

Tamoxifen (Sigma-Aldrich) was dissolved in corn oil to a final concentration of 10 mg/mL. Tamoxifen (10 μL/g) was injected intraperitoneally at three different developmental time points, e.g., at postnatal day (P)1-2, P15-17 and P46-48. For P1-2 injections, tamoxifen was injected intraperitoneally into the mother of the pups when the pups were P1-2 and received tamoxifen via mother milk. For P15-17 and P46-48, tamoxifen was injected intraperitoneally directly into the pups for three consecutive days.

Electron microscopy and morphometric analysis

Mice (P56, n=3-4) under deep anesthesia were perfused transcardially with 0.1 M phosphate buffered saline (PBS; pH 7.4) containing 0.1% heparin followed by freshly prepared cold fixative solution (4% paraformaldehyde (PFA) in 0.1 M PBS, pH 7.4). Brains were removed, post-fixed overnight in fixative solution at 4 °C, rapidly frozen in powdered dry ice. 50 μm-thick sagittal sections were sliced using a cryostat (-20 °C), rinsed three times and dehydrated with ethanol. After embedding in Epon, ultra-thin sections (~90 nm) were cut, collected on formvar coated single slot grids, and stained with a 1% aqueous uranyl acetate solution and subsequently with lead citrate. Digital images were obtained with a JEOL 1010 electron microscope (Peabody, MA).
Morphometric analysis of myelinated axons in the corpus callosum

For each myelinated axon in the corpus callosum, the g-ratio was calculated by dividing the axonal diameter (defined by the inner limit of the myelin sheath) by the total fiber diameter (defined by the outer limit of the myelin sheath).

Ultrastructural analysis of asymmetric synapses in the CA1 region of the hippocampus

Only synapses with clear post- and presynaptic properties were selected for analysis. For each condition, postsynaptic density and active zone length (nm), the number of docked and undocked vesicles, as well as vesicle cluster size (nm²) were measured as described previously [163]. Vesicles were characterized as docked when no separation was detectable between the vesicle membrane and the active zone. The vesicle cluster size was defined as the area within the presynaptic terminal that contained all synaptic vesicles. A total of 40-50 synapses per animal were analysed.

Immunohistochemistry

Mice (P56) were deeply anesthetized and perfused transcardially with 0.1 M PBS (pH 7.4) containing 0.1% heparin followed by freshly prepared cold fixative solution composed of 4% PFA in 0.1 M PBS (pH 7.4). Brains were removed and post-fixed overnight in fixative solution at 4 °C. After rapidly freezing the brains in powdered dry ice, 50 μm-thick sagittal sections were sliced using a cryostat (-20 °C). Sections were rinsed three times and stored in 50 μm-thick sagittal sections in 0.1 M PBS (pH 7.4) with 0.1% NaN₃ at 4 °C or with 0.1% anti-freeze solution at -20 °C until use. For immunostainings, sections were rinsed four times in 0.1 M PBS for 10 min, blocked for 30 min in blocking solution (0.1 M PBS containing 5% normal goat serum, 2.5% bovine serum albumin and 0.2% Triton-X-100), and subsequently incubated overnight with primary antibodies in blocking solution with mild shaking at 4 °C. The sections were rinsed four times, followed by incubation with secondary antibodies in blocking solution for 2 h at room temperature. Sections were rinsed again four times and subsequently mounted in Vectashield mounting medium including DAPI as a nuclear dye (Vector Laboratories) on glass slides. The following primary antibodies were used: goat anti-Olig2 (1:30, Millipore), mouse anti-NeuN (1:2.000, Millipore), rabbit-anti-FASN (1:1.000, Abcam) and mouse anti-GFAP (1:500, Sigma), the latter only when staining slices of control mice. Secondary antibodies were from Molecular probes: Alexa fluor 488/568-conjugated goat anti-mouse (1:400), Alexa fluor 488-conjugated goat anti-rabbit (1:400), Dyl488-conjugated donkey anti-goat (1:200). Sections were examined on a Leica DMI8 microscope using a 40x objective, sampled with a Leiko DFC3000G camera under fluorescent illumination. Cell density was determined using Leica Microsystems LAS AF Lite software.
Statistical analysis
Data are presented as mean ± SEM. Statistical differences were analyzed using Student’s t-test.

RESULTS
To inactivate lipid synthesis in astrocytes in a temporally controlled manner, Glast-CreERT2-tdT-SCAP mice were generated, which are referred to as Glast-SCAP mice. For this, SCAPloxP mice were crossed with mice expressing the tamoxifen-inducible CreERT2 under the transcriptional control of the astrocyte-specific Glast promoter, and with mice expressing the td-Tomato reporter in order to visualize recombined cells (Figure 1A-B). Using these Glast-SCAP mice, recombination was induced at various time points during postnatal developmental, which prevents potential neural progenitor perinatal and early postnatal targeting [202]. Here, SCAP deletion was postnataally induced at P1-P2 (before synaptogenesis and myelination), at P15-P17 (after the peak of synaptogenesis and at the peak of myelination) and in young adults at P46-P48 (during synapse and myelin maintenance) (Figure 1C-D).

Postnatal tamoxifen-induced recombination in peripheral tissue
The fact that the glutamate-aspartate transporter (Glast) is widely expressed by astrocytes [204], makes it one of the most well-known astrocyte markers and is therefore extensively used for mouse lines allowing selectively targeting of astrocytes in vivo [202]. Surprisingly, during our general investigation of the Glast-SCAP mice, we found that tamoxifen injections at P1-2 resulted in expression of the td-Tomato reporter in peripheral organs at P56, such as the salivary glands and pancreas, meaning that tamoxifen also induced Cre-mediated recombination in these tissues (Figure 2A, Table 1). Peripheral recombination in Glast-SCAP mice (P56) was strongly reduced but still present when tamoxifen was injected at later developmental time points, e.g., after the peak of synaptogenesis and at the peak of myelination (P15-17; Table 1) and during synapse and myelin maintenance (P46-48; Figure 2B, Table 1). Tamoxifen injections did not result in peripheral recombination in control mice, which do not express the CreERT2 recombinase, leading to normal astrocyte SCAP-SREBP-mediated lipid synthesis (data not shown).
Figure 1. Generation of tamoxifen-induced SCAP deletion in astrocytes at different developmental time points. (A) Schematic representation of the genetics used to generate Glast-CreERT2-tdT-SCAP mice, referred to as Glast-SCAP mice: Glast-CreERT2 × SCAP-floxed × Rosa26-tdTomato. (B) Without tamoxifen (TMX), Cre recombinase fused to a mutated form of the human estrogen receptor (ERT2) is bound to heat shock protein (Hsp90), keeping CreERT2 in the cytoplasm. (C) Upon intraperitoneal injections of tamoxifen, CreERT2 becomes activated in Glast+ cells and translocates to the nucleus. In the nucleus, CreERT2 recognizes loxP sites leading to recombination-mediated deletion of the first exon of SCAP and removal of the flanked stop cassette, allowing the expression of the td-Tomato reporter gene. In this way, tamoxifen injections result in inactivation of SCAP and the expression of the td-Tomato reporter in Glast+ cells. (D) Timeline of tamoxifen injections at three different developmental time points. Tamoxifen was either injected intraperitoneally at P1-2 (before synaptogenesis and myelination), at P15-17 (after the peak of synaptogenesis and at the peak of myelination) or P46-48 (during synapse and myelin maintenance). Analysis was performed when mice were at P56. Timeline was adapted from [141].
Figure 2. Phenotype of Glast-SCAP mice (at P56) injected with tamoxifen at P1-2 or P46-48. (A) Cre-mediated recombination in Glast-SCAP mice (at P56) after tamoxifen injections when the pups were at P1-2. Shown are the skin abnormalities of the tail, ears and paws. (B) Cre-mediated recombination in Glast-SCAP mice (at P56) after tamoxifen injections at P46-48. Shown is a normal skin phenotype and td-Tomato expression in salivary glands and pancreas.

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Table 1. Phenotype of Glast-SCAP mice (at P56) injected with tamoxifen at either P1-2, P15-17 or P46-48. P1-2 tamoxifen-injected Glast-SCAP mice showed reduced (↓) survival and activity, their fur appeared greasy/ungroomed and strong (+++) td-Tomato expression was observed in peripheral organs, such as salivary glands and pancreas. P15-17 and P46-48 tamoxifen-injected Glast-SCAP mice had normal survival and activity, their fur and skin appeared normal and they showed moderate (+) levels of td-Tomato expression in salivary glands and pancreas.
P1-2 tamoxifen-injected Glast-SCAP mice showed reduced survival and activity as well as a greasy/ungroomed fur and skin abnormalities of the paws, tail and ears (Figure 2A), which appeared normal in tamoxifen-injected control mice or when SCAP mutation was induced at P15-17 or P46-48 (Table 1). These observations showed that well-being of Glast-SCAP mice is compromised after tamoxifen-induced recombination at P1-2, while being unaffected when induced at later developmental time points. To determine the effect of SCAP mutation on myelination and synapse function, we therefore continued analyzing mice with tamoxifen-induced SCAP mutation at P15-17, just after the peak of synaptogenesis and at the peak of myelination.

![Figure 3](image)

**Figure 3.** Cellular expression analysis of the recombination-reporter protein td-Tomato in different brain regions of Glast-SCAP mice (at P56) injected with tamoxifen at P15-P17. (A) Colocalization of td-Tomato (tdT; in red) with astrocytes (GFAP; in green), oligodendrocytes (Olig2; in green) or neurons (NeuN; in green) in the corpus callosum, as depicted. (B) Colocalization of td-Tomato (tdT; in red) with astrocytes (GFAP; in green), oligodendrocytes (Olig2; in green) and neurons (NeuN; in green) in the CA1 region of the hippocampus. Arrowheads denote cells that show co-expression of tdT with cell-type specific markers. Scale bar, 25 μm.
Figure 4. Fatty acid synthase expression in astrocytes of Glast-SCAP control or mutant mice (at P56) injected with tamoxifen at P15-P17. (A) Expression of fatty acid synthase (FASN; in green) in astrocytes in the corpus callosum of tamoxifen-injected control (Ctrl) or Glast-SCAP mutant mice (Mut). Bar graph shows the percentage of FASN-positive astrocytes in control (GFAP+FASN+) and Glast-SCAP mice (tdT+FASN+), FASN-negative astrocytes (GFAP/tdT+FASN-) or FASN-positive non-astrocyte cells (GFAP/tdT-FASN+). (B) FASN (in green) expression in astrocytes in the CA1 region of the hippocampus of Ctrl or Mut mice. Astrocytes are visualized by GFAP staining (red, for Ctrl mice) or tdT expression (red, for Mut mice). Arrowheads denote FASN+ astrocytes, asterisks indicate FASN+ cells that are negative for GFAP or tdT (scale bar, 25 μm). Data are presented as mean ± SEM. ***p<0.001 using Student’s t-test.

Postnatal tamoxifen-induced inactivation of SCAP results in impaired lipid synthesis, specifically in astrocytes, leading to impaired myelination and changes in synapse ultrastructure

To examine whether tamoxifen injections resulted in recombination in astrocytes specifically, we analyzed td-Tomato expression in the corpus callosum and the hippocampus (CA1). Immunohistochemical analysis revealed that Glast-SCAP mice injected with tamoxifen at P15-17 had td-Tomato reporter gene expression in the large majority of GFAP+ astrocytes in both the hippocampus and corpus callosum (Figure 3A-B and S1A). Virtually no expression
was found in Olig2+ oligodendrocytes or NeuN+ neurons (Figure 3A-B and S1B-C). Moreover, a strong reduction in the expression of fatty acid synthase (FASN), a SREBP target gene [124], was observed in astrocytes in the hippocampus and corpus callosum of Glast-SCAP mutant mice (Figure 4A-B). These results demonstrate that tamoxifen-induced SCAP mutation results in impaired lipid synthesis specifically in astrocytes.

To establish the role of postnatal astrocyte lipid synthesis in myelination, P15-17 tamoxifen injected mice (P56) were analyzed using electron microscopy (EM). The formation of myelin sheaths, which starts in the second postnatal week, is most active during postnatal day 20 [141] and requires extraordinarily high levels of lipid synthesis [179]. Inactivated astrocyte lipid synthesis at P15-17, at the peak of myelination, resulted in a hypomyelinated corpus callosum (Figure 5A), which predominantly affected the small caliber fibers (Figure 5B). In contrast, axonal diameter distribution was not affected (Figure 5C).

**Figure 5.** Electron microscopy analysis of corpus callosum myelination of Glast-SCAP control or mutant mice (at P56) injection with tamoxifen at P15-P17. (A) EM analysis of corpus callosum myelination in cross-sections of either tamoxifen-injected control (Ctrl) or Glast-SCAP mutant mice (Mut) (left two panels). The relation between axon diameter (x) and g-ratio (y) was y = 0.1308x + 0.6872 for Ctrl and y = 0.1022x + 0.7408 for Mut, with coefficients of determination $R^2 = 0.32986$ (Ctrl) and $R^2 = 0.29418$ (Mut) (right panel). (B) Morphometric analysis of axons in the corpus callosum of Ctrl and Mut mice showing g-ratio per class of axonal diameter for myelinated axons. (C) Axonal size distribution for both myelinated and non-myelinated axons. *p<0.05 using Student’s t-test.

This suggests that myelin membrane synthesis is reduced in Glast-SCAP mice, causing persistent CNS hypomyelination. Next, the ultrastructure of asymmetric synapses in the CA1
region of the hippocampus was determined in adult Glast-SCAP mice that were injected with tamoxifen at P15-P17 (Figure 6A). We observed a reduction of both the length of the postsynaptic density (PSD) and the presynaptic active zone (AZ) (Figure 6B), while the number of vesicles that are in close proximity of the AZ and ready for release, i.e., the docked vesicles, as well as the total number of vesicles and the size of the vesicle cluster, were not affected (Figure 6C-D).

Taken together, compromised astrocyte lipid metabolism, when induced after the peak of synaptogenesis but during the active phase of myelination, leads to persistent CNS hypomyelination and small changes in synapse ultrastructure.

Figure 6. Electron microscopy analysis of hippocampal synapses of Glast-SCAP control or mutant mice (at P56) injected with tamoxifen at P15-P17. (A) Representative example of Glast-SCAP control (Ctrl) or mutant (Mut) synapses in the CA1 region of the hippocampus. (B) Size of the postsynaptic density and presynaptic active zone. (C) Number of docked vesicles (vesicles close to the active zone) and of undocked and total vesicles. (D) Size of the cluster containing all vesicles. *p<0.05 using Student’s t-test.
DISCUSSION

Here we generated a mouse model that expresses the tamoxifen-inducible CreERT2 under transcriptional control of the astrocyte-specific Glast promoter and we investigated the effects of postnatal SCAP deletion specifically in astrocytes. We observed that tamoxifen-induced SCAP deletion at P1-2 induced strong peripheral recombination and compromised animal well-being, while this was mainly unaffected when SCAP was deleted later during development (P15-17) or in young adults (P46-48). We found that inactivation of the SCAP-SREBP-pathway in astrocytes at P15-P17 (after the peak of synaptogenesis and at the peak of myelination) resulted in reduced astrocyte lipid synthesis, leading to hypomyelination and changes in synapse ultrastructure.

Glast-CreERT2-SCAP mice as a tool to study temporal inactivation of astrocyte lipid metabolism

We previously reported that SCAP deletion in astrocytes during early embryonic development, using GFAP-SCAP mice, resulted in microcephaly and neurological impairments, including progressive motor deficits and reduced anxiety [56], which was accompanied by impaired presynaptic terminal development and synaptic plasticity [163]. The GFAP-SCAP mouse line is a powerful tool to study the effect of inactivated SCAP-SREBP-mediated lipid biogenesis in astrocytes during embryonic development, however, the specific effects of SCAP deletion postnatally remains unknown. Furthermore, it has been shown that the GFAP promoter predominantly targets the majority of astrocytes, but potentially also some neural progenitor cells [125,205]. It should be noted that although the level of lipid synthesis is very low in neurons when compared to astrocytes [33,34,135], and hippocampal neurons do not express SREBP in vitro [163], the possibility that a small subset of neurons were targeted cannot not be excluded. To investigate the effects of postnatal astrocyte SCAP deletion and to prevent potential neural progenitor targeting, we generated and analyzed a mouse model in which tamoxifen induced the deletion of SCAP in cells expressing the astrocyte-specific Glast promoter. Glast, playing a crucial role in glutamatergic neurotransmission by removing glutamate from the extracellular space [206], is widely expressed by astrocytes and is a commonly used astrocyte-specific marker. Accordingly, transgenic mouse lines using the Glast promoter, such as the Glast-CreERT2 mice, are used in many studies investigating astrocyte function in the brain (reviewed in [207]).

Here we observed that tamoxifen-induced recombination during early postnatal development (P1-2) was not restricted to the CNS, but was also present in specific peripheral organs. Our finding that Cre-mediated recombination took place in the salivary glands and pancreas is in line with a recent study showing Glast expression in these peripheral organs [208]. However, this study also reported Glast mRNA and protein expression in epithelial
cells, lymphatic organs and glands, showing that Glast is widely distributed outside the CNS [208], although we did not observe td-Tomato expression in peripheral tissues other than salivary glands and pancreas. Similarly, a study reporting Cre-mediated recombination in the Glast-CreERT2 mouse line showed strong targeting of the spleen and skin but not other peripheral tissues expressing Glast [209]. Therefore it is likely that peripheral Glast expression, in combination with SCAP expression outside the CNS, might have resulted in reduced lipid synthesis in cells also outside the CNS. It is known that the skin has a large capacity for lipid synthesis in order to maintain a permeability barrier that protects against transepidermal loss of water and electrolytes [210]. Downregulation of lipid synthesis in the skin might therefore have large consequences. Indeed, it was reported that a skin-specific deletion of stearoyl-CoA desaturase-1 (SCD1), a fatty acid desaturase under the control of SREBP-1c and found to be downregulated in GFAP-SCAP mice [56], is linked to atopic dermatitis [210], a type of skin inflammation that is characterized by a red, swollen, dry and cracked skin. The skin phenotype of Glast-SCAP mice injected with tamoxifen at P1-2 seems consistent with the skin phenotype of SCD1 knockout mice. Furthermore, tamoxifen-induced recombination in the pancreas might have affected pancreas function and might result in changes in the digestive system or glucose homeostasis. It has been demonstrated that SREBP-1c is expressed in β-cells of the pancreatic islets and that SREBP-1c deletion enhanced basal and glucose-induced insulin secretion in β-cells [211,212]. Unbalanced glucose homeostasis leads to impaired systemic metabolic responses but might also affect neuronal brain activity since glucose is transported through the blood-brain barrier [213]. Moreover, unbalanced glucose homeostasis may affect peripheral lipid levels. Together with our recent finding that dietary lipids are able to affect CNS myelination and synapse maturation [78], this indicates that lipids from the periphery are able to modulate brain function and that reduced lipid synthesis in peripheral tissues, due to tamoxifen-induced peripheral recombination in Glast-SCAP mice, might affect processes in the brain. Therefore, given that peripheral recombination was much less present when induced later during development (P15-17) or in young adults (P46-48), we continued analyzing mice with tamoxifen-induced SCAP mutation at P15-17, to establish the role of astrocyte-specific lipid synthesis on synapse maintenance and the formation of myelin membranes. To establish the role of astrocyte lipid synthesis in synapse function and myelin maintenance, future experiments might be performed with young adult mice, in which SCAP is inactivated at P46-48.

Postnatal inactivation of astrocyte SCAP interferes with myelination and synapse ultrastructure

In this study, we found that SCAP deletion at P15-17 inhibited lipogenic gene expression in the CNS, specifically in astrocytes. This inactivation of astrocyte lipid synthesis at P15-17
resulted in a reduction in the size of both the presynaptic active zone and postsynaptic density. This indicates that astrocyte SCAP deletion after the peak of synaptogenesis might interfere with pre- and postsynaptic terminal size, since the size of the postsynaptic density and active zone positively correlates with spine size and bouton size, respectively [142,214]. This might result in decreased synaptic transmission, given that a reduced spine head size is associated with immature synapses [144] and correlates with a decreased number of postsynaptic receptors [215,216].

We found that the number of docked, undocked and total number of vesicles was unaffected in Glast-SCAP mice, suggesting that postnatal SCAP deletion at P15-17 does not interfere with the synaptic vesicle cycle. We previously found that deletion of SCAP during early embryonic development resulted in a smaller docked vesicle pool and total vesicle pool, whereas the size of the presynaptic active zone as well as the size of the postsynaptic density and PSD-95 protein levels were not affected [163]. These opposing results suggest that the time point at which SCAP is deleted in astrocytes determines which different developmental phases are interfered with. Indeed, it is known that synaptogenesis starts at the first postnatal week and continues for three weeks, while peaking during postnatal day 10 [141]. During synaptogenesis, presynaptic terminal differentiation, which is characterized by the formation and recycling of synaptic vesicles, precedes postsynaptic terminal differentiation [217]. Therefore, it is plausible that interfering with astrocyte lipid synthesis around P15-17, when most synapses have been formed, has only small effects on synapse ultrastructure, compared to SCAP deletion during embryonic development, when the brain is highly active in neuro- and synaptogenesis [141]. Indeed, interference with astrocyte lipid metabolism after the process of synaptogenesis causes impaired postsynaptic terminal differentiation, a process that follows presynaptic differentiation and takes place during a later stage of synaptogenesis [217]. Additional experiments focusing on synapse number, morphology and function will be necessary for understanding the role of postnatal astrocyte lipid metabolism in synapse formation, maturation and function.

Finally, astrocyte SCAP deletion at P15-17 also strongly reduced membrane myelination in the corpus callosum, suggesting that inactivating astrocyte lipid synthesis at the peak of myelination when myelination is most active [141], has profound effects on this process. This is in line with our recent observations that embryonic SCAP deletion, using GFAP-SCAP mice, resulted in persistent CNS hypomyelination [78], showing that astrocyte lipids contribute to myelination during development.

In summary, the Glast-CreERT2-SCAP mouse is a powerful tool to dissect the role of astrocyte lipid metabolism during specific developmental time points. Postnatal inactivation of astrocyte SCAP-SREBP-mediated lipid biogenesis, after the peak of synaptogenesis and at the peak of myelination, resulted in reduced lipid biogenesis specifically in astrocytes, leading
to hypomyelination and small changes in synapse ultrastructure. This inducible mouse model, in which astrocyte lipid metabolism is inactivated during postnatal development, may have important implications for the understanding and treatment of neurological disorders with a postnatal onset of compromised lipid metabolism, such as Alzheimer’s disease and Huntington’s disease.

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SUPPLEMENTARY FIGURES

Figure S1. Expression of recombination-reporter protein td-Tomato in different cell types in the hippocampus of Glast-SCAP mice (at P56) injected with tamoxifen at P15-P17. Td-Tomato expression (tdT; in red) in (A) astrocytes (GFAP; in green), (B) oligodendrocytes (Olig2; in green) and (C) neurons (NeuN; in green) in the CA1 region of the hippocampus (scale bar, 25 μm).