SCAP in glia-neuron interactions

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CHAPTER 5

GFAP-SCAP mutant mice are impaired in LTP and contextual fear memory

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Abstract

The brain has a remarkably different lipid composition than other organs; it is highly enriched in poly-unsaturated fatty acids (PUFA) and cholesterol, both major constituents of synaptic membranes. Unlike glial cells, neurons have a poor capacity to synthesize lipids, and specifically astrocytes have been proposed, based on *in vitro* studies, to actively supply lipids to neurons and thereby regulate synapse formation and function. However, the contribution of astrocyte lipid synthesis to synaptic function *in vivo* is not clear. To investigate this, we analysed mice in which the sterol regulatory element-binding protein (SREBP) cleavage-activating protein (SCAP) was deleted from GFAP-cre expressing cells. Next to astrocytes, also a large population of granular cells in the hippocampus is targeted in these mice and consequently is defective in lipid synthesis. SCAP mutant mice showed strongly reduced hippocampal LTP and impaired contextual fear memory, however, had only small changes in synaptosomal lipid composition. Treatment with a high fat diet ameliorated the compromised synaptosomal lipid composition and improved, hippocampal LTP of SCAP mutant mice, although not significantly. Together, these data show that hippocampal function is dependent on local SREBP dependent lipid synthesis, and indicate that functional impairment of the hippocampus by lipid synthesis deficiencies may be ameliorated by dietary lipid supplementation.

Introduction

Lipids are vital components of synaptic membranes and have been shown to influence brain function in many different ways (Piomelli et al. 2007). The synaptic membrane is enriched in cholesterol and unsaturated fatty acids, which together determine the fluidity of the membrane, affect vesicle formation and fusion, regulate ion channel function and form specialized microdomains that contribute to cellular communication (for review see Allen et al. 2007). In addition, many lipids are precursors of signalling molecules (*e. g.*, neurosteroids, prostaglandins) (Belelli and Lambert 2005) or act as second messengers (*e. g.* arachidonic acid) that initiate intracellular cascades (Piomelli 1993; Piomelli et al. 2007). Consequently, perturbed lipid metabolism affects synaptic function (Hering et al. 2003; Kotti et al. 2008; Jang et al. 2009) and thereby learning, memory and behavior (Frye and Sturgis 1995; Kotti et al. 2006).

In vitro studies have shown that astrocytes support neurite outgrowth and the formation, maturation and maintenance of synapses of cocultured neurons (Nagler et al. 2001; Slezak and Pfrieger 2003). In a series of studies, Tabernero and Medina have demonstrated that astrocytes synthesize and release oleic acid, which stimulates neuronal differentiation (Tabernero et al. 2001; Medina and Tabernero 2002; Tabernero et al. 2002; Polo-Hernandez et al. 2010). Moreover, Moore and co-workers demonstrated that astrocytes, unlike neurons, are active in polyunsaturated fatty acids (PUFA) synthesis (Bilunas and Moore 2002). PUFAs strongly stimulate neurite outgrowth (Darios and Davletov, 2006) and affect synaptic transmission. For instance, docosahexanoic acid (DHA) was demonstrated to modulate ion currents in isolated hippocampal neurons (Vreugdenhil et al. 1996), and arachidonic acid (AA) was reported to stimulate neurotransmitter release via direct binding to syntaxin, a component of the vesicle release machinery (Connell et al. 2007). Besides oleic acid and PUFAs, also cholesterol is synthesized and released in complex with apolipoprotein E (ApoE) by astrocytes, and is a key player in synapse formation and function (Mauch et al. 2001). Cholesterol is a major component of neuronal membranes and it forms specialized microdomains, called lipid rafts, which are required presynaptically for synaptic vesicle formation (Thiele et al. 2000) and postsynaptically for the clustering and stability of neurotransmitter receptors (Allen et

al. 2007). Together, these findings argue for a prominent role of astrocyte-derived lipids in synapse development and function. In addition, it may be speculated that via similar mechanisms, astrocytes potentially regulate synaptic plasticity in the adult brain. The regulation of astrocyte synthesis of unsaturated fatty acids and cholesterol requires the SREBP transcription factors, which are activated by the sterol sensor SCAP (Camargo et al. 2012). Thus, SCAP/SREBP in astrocytes may function in the controlled supply of cholesterol and fatty acids to the synapse and thereby contribute to synaptic function.

We have previously shown that SCAP deletion in astrocytes leads to paroxysmal dyskinesia, premature death and a series of neurological impairments (Camargo et al. 2012). Here we studied the role of hippocampal SCAP/SREBP in hippocampal function, and the effect of exogenous lipid supply under conditions that SCAP/SREBP action is compromised. SCAP mutants had reduced fear conditioning, reduced LTP, and showed small changes in fatty acid composition of hippocampal synaptosomes. Interestingly, both LTP and synaptic lipid abnormalities were ameliorated by a high fat diet. Thus, we conclude that local SCAP/SREBP dependent lipid synthesis is necessary for proper hippocampal function and that components of a high fat diet may be an appropriated treatment for hippocampal dysfunctions associated with compromised lipid metabolism.

Results

We previously reported that GFAP-SCAP mutant mice have motor dysfunction and premature death (Camargo et al. 2012). It has been shown that the GFAP promoter targets not only astrocytes but also a subset of neurons due to the role of GFAP expressing cells as progenitor for neurons (Fraser et al. 2004). In particular, this has been shown for the hippocampus where neurogenesis takes place during brain development and in the adult brain. However, in the mouse line we used in this study (Bajenaru et al. 2002), glia targeting is less widespread as radial glia is not affected (Bottelbergs et al. 2010). We therefore attribute changes seen in these experiments to both astrocytes and to a small extent to neurons defective in lipid metabolism.

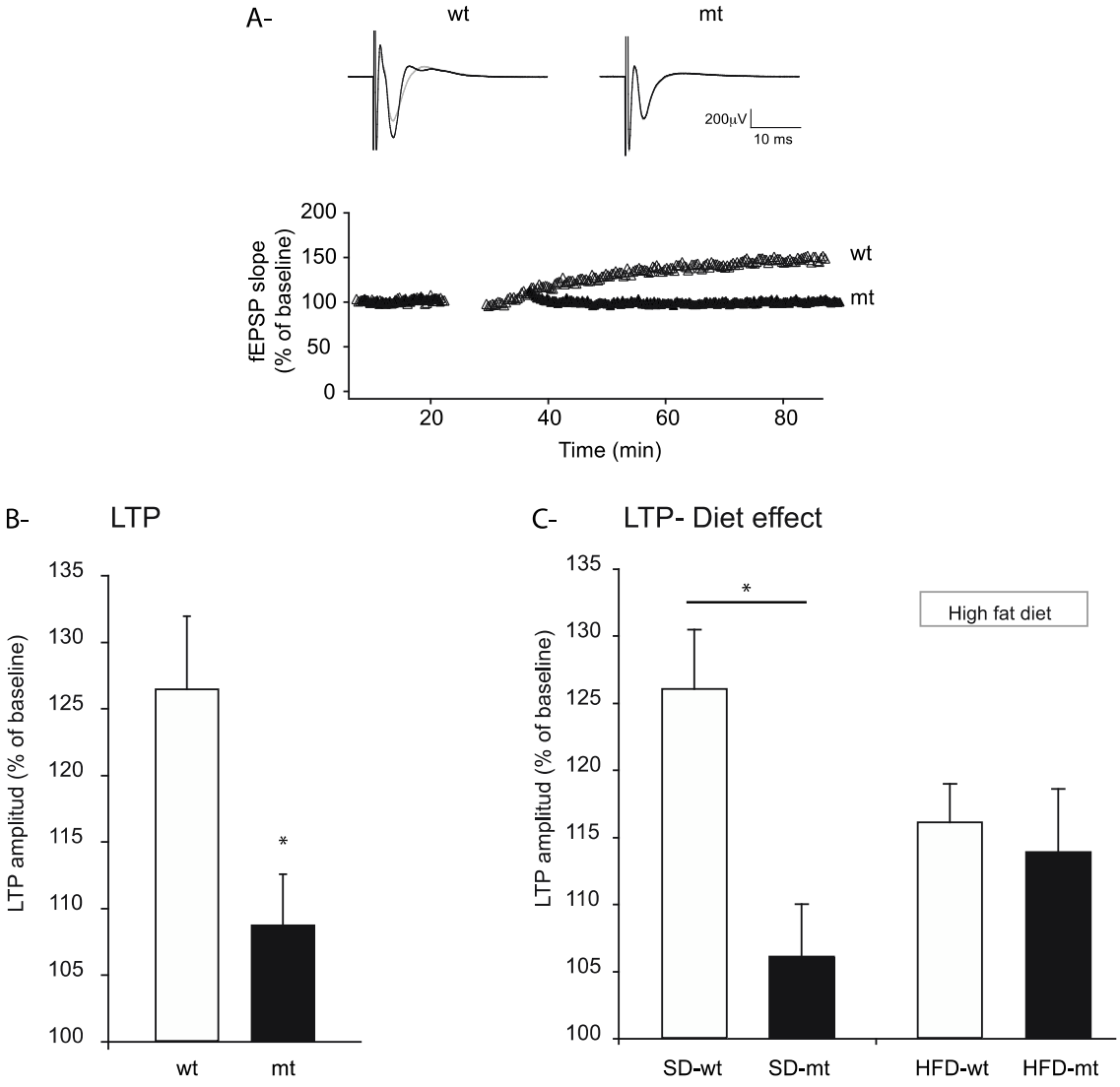

Figure 1. Hippocampal LTP is impaired in SCAP mutants and improved by a high fat diet. A) Upper panel: representative example of recorded potentials in hippocampal slices of wild-type (wt) and mutant (mt) mice immediately after stimulation (grey line) and around 20 minutes after stimulation (black line), note that no difference is found in the mutant. Lower panel: representative example of LTP in a hippocampal slice of a wild-type (open triangles) and a SCAP mutant mouse (black triangles). B) Histograms showing the amplitude of induced LTP recorded in slices of wild-types (white bar, n= 15 slices) and mutants (black bar, n= 18 slices). C) Independent experiment showing that a high fat diet improves LTP in mutants (number of slices: SD-wt, 20; SD-mt, 15; HFD-wt, 21 and HFD-mt, 16). Data are presented as mean ± SEM. (MANOVA and Tukey post-hoc test, *= $p < 0.05$).

SCAP mutants have reduced hippocampal LTP induction, which is improved by a high fat diet

To determine whether SCAP mutants have affected hippocampal synaptic plasticity, we measured long-term potentiation (LTP) of local field potentials. The induction of LTP in Schaeffer collaterals from CA3 to CA1 was analysed in SCAP mutant and

wild-type mice. For this, field EPSPs were recorded in hippocampal coronal slices before and after induction of LTP by theta-burst stimulation (TBS) using multi electrode grids. In SCAP mutants, TBS resulted in a smaller increase in synaptic responses (+8.67 % ± 3.9) than in the littermate controls (+26.4 % ± 5.5) (Fig. 1B, example traces in Fig. 1A).

We previously showed that feeding SCAP mutants with a high fat diet (HFD) increased survival, and delayed the onset of paroxysmal dyskinesia (Camargo et al. 2012). Moreover, it improved myelination, which was strongly reduced in mutants (see Chapter 4). To determine whether a HFD is also successful in improving the compromised LTP in SCAP mutant mice, we compared LTP amplitude in wild-types and mutants fed with the standard diet (SD) versus the HFD. Again, mutants on SD were found to have decreased induction of LTP (WT: +24.3 % ± 4.4 vs MT: +5.3% ± 3.9). Interestingly, we found that the HFD modestly improved LTP in SCAP mutants (to 14 % ± 4.7), although statistically not significant. We also found that LTP tends to be lower in wild-types in HFD compared to wild-types in SD (Fig. 1C). Thus, we conclude that SCAP deletion impairs LTP induction and that external lipid supply may recover compromised LTP in SCAP mutant mice.

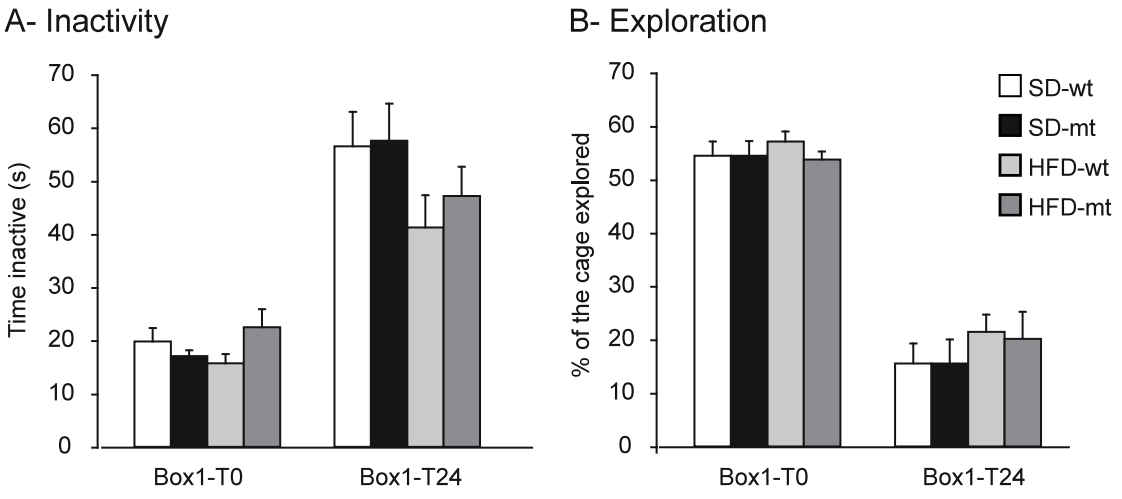

Figure 2. Assessment of contextual fear memory. Mice were placed in Box1, allowed to explore for 3 min and then received a foot shock. Mice were placed back in their home cage and after 24 hours placed again in Box1 and allowed to explore for 3 min. Shown are the percentages of inactivity (A) and exploration (B) before delivery of the electric shock (Box1-T0) and after 24 hours in Box1 (Box1-T24) of wild-type (wt) and mutant (mt) mice on standard diet (SD) or high fat diet (HFD). Data are presented as mean ± SEM. (MANOVA and Tukey post-hoc test; n=5-8).

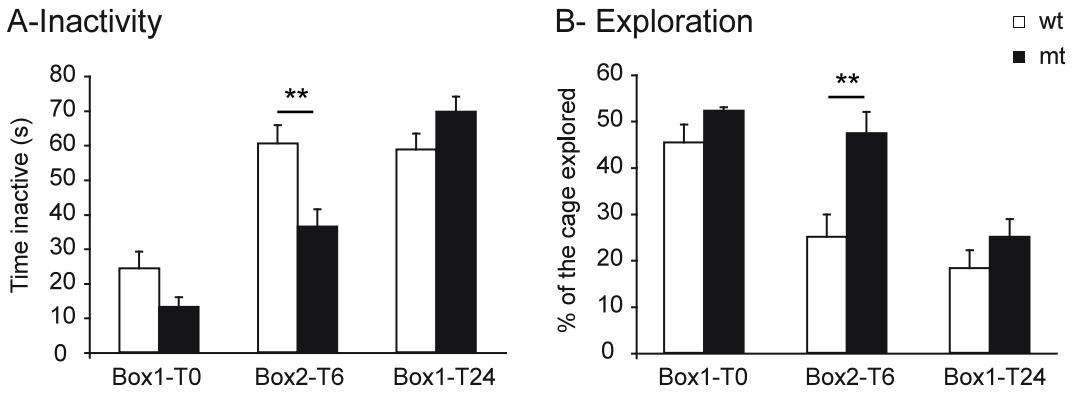

Figure 3. Assessment of contextual fear memory in two slightly different environments. Mice were placed in Box1, allowed to explore for 3 min, then received a foot shock, and were subsequently placed back in their home cage for 6 hours and then exposed to a different box (Box2), for 3 min. Next, mice were placed back in their home cage and after 24 hours, mice were placed again in Box 1 and allowed to explore for 3 min. Shown are the percentages of inactivity (A) and exploration (B) before delivery of the electric shock (Box1-T0), after 6 hours in Box2 (Box2-T6) and after 24 hours in Box1 (Box1-T24) for wild-type (wt) and mutant (mt) mice. Data are presented as mean ± SEM. (ANOVA + Tukey post-hoc test, **= $p < 0.01$; n=12).

SCAP mutants have impaired contextual learning

The hippocampus plays an important role in contextual fear memory (Mamiya et al. 2009). To determine whether the observed reduction in LTP is associated with impaired memory in SCAP mutants, we measured contextual fear conditioning in these animals and used it under the conditions of a standard diet and a high fat diet. Mice were placed in a novel environment (Box1), and a foot shock was delivered after 3 minutes (Box1-T0). Twenty-four hours after the shock the mice were placed in the same environment (Box1-T24), and fear memory was determined by comparing inactivity (Fig. 2A). Cage exploration was also measured at T0 and T24 (Fig. 2B). When re-exposed to the shock cage (Box1-T24), mice of all experimental groups had a large increase in inactivity and they showed less exploration, while no differences were found between wild-type and mutant mice. The HFD decreased inactivity, although not significantly (wt-SD *vs* wt-HFD, p= 0.08; mt-SD *vs* mt-HFD, p= 0.07), suggesting that memory is affected both in wild-type and mutant mice fed with this diet. These results indicate that SCAP deletion in astrocytes does not alter fear conditioning in animals tested 24 hours after exposure, but that as with LTP, the use of the diet has a negative effect on memory in both genotypes.

Next, we tested a different group of SCAP mutants and wild-type animals for contextual learning in an adapted fear-conditioning paradigm, using application of a foot shock in a novel environment (Box1), and testing of the animals 6 hours after the shock in a slightly changed environment (Box2). Whereas both wild-type and mutant mice reacted on the foot shock (Box1-T0), only wild-types showed an increase in activity when placed back in a slightly changed environment after 6 hours (Box2-T6). When the animals were placed back in Box1 24 hours after the shock (Box1-T24) mutants showed the same degree of inactivity as wild-types (Fig. 3A). Similarly, exploration was not reduced at 6 hours in mutants but it was at 24 hours (Fig. 3B). Thus, we conclude that contextual memory of SCAP mutants is affected when contextual learning is more challenging.

SCAP mutants have small changes in hippocampal synaptic lipid composition

We previously found that lipid composition of total brain extracts was altered in SCAP mutant mice (Camargo et al. 2012). To determine whether these changes in lipid composition were also localized to synapses, we analyzed cholesterol and fatty acid composition of fractions highly enriched in hippocampal synaptosomes. We found no prominent changes in the levels of cholesterol in SCAP mutant synaptosomes (Fig. 4A). Moreover, feeding the mice with a high fat enriched diet did not induce any changes in synaptosome cholesterol levels, not for mutants nor for wild-type mice. Also, the total fatty levels were not affected by SCAP deletion or by the high fat diet (Fig. 4B). However, analysis of the percentage of different fatty acids revealed an accumulation of stearic acid in mutant mice (Fig. 4C and 4E), and possibly also of linolenic acid (C18:3ω3; p= 0.15), and linoleic acid (C18:2ω6; p= 0.06) and stearidonic acid (C18:4ω3; p= 0.09), an intermediate product in the synthesis of docosahexanoic acid (DHA) (Fig. 4C and 4D). The levels of oleic acid, DHA and arachidonic acid (AA) were not affected. Interestingly, treatment of mutant animals with HFD normalized the abnormal levels of stearic acid and that of the precursors for DHA (C18:4 and C18:3). Together, we conclude that SCAP deletion affected the levels of a few specific fatty acids, *e. g.* stearic acid, stearidonic acid and linolenic acid, in hippocampal synaptosomes and that treatment with HFD resulted in the normalization of these to wild-type levels.

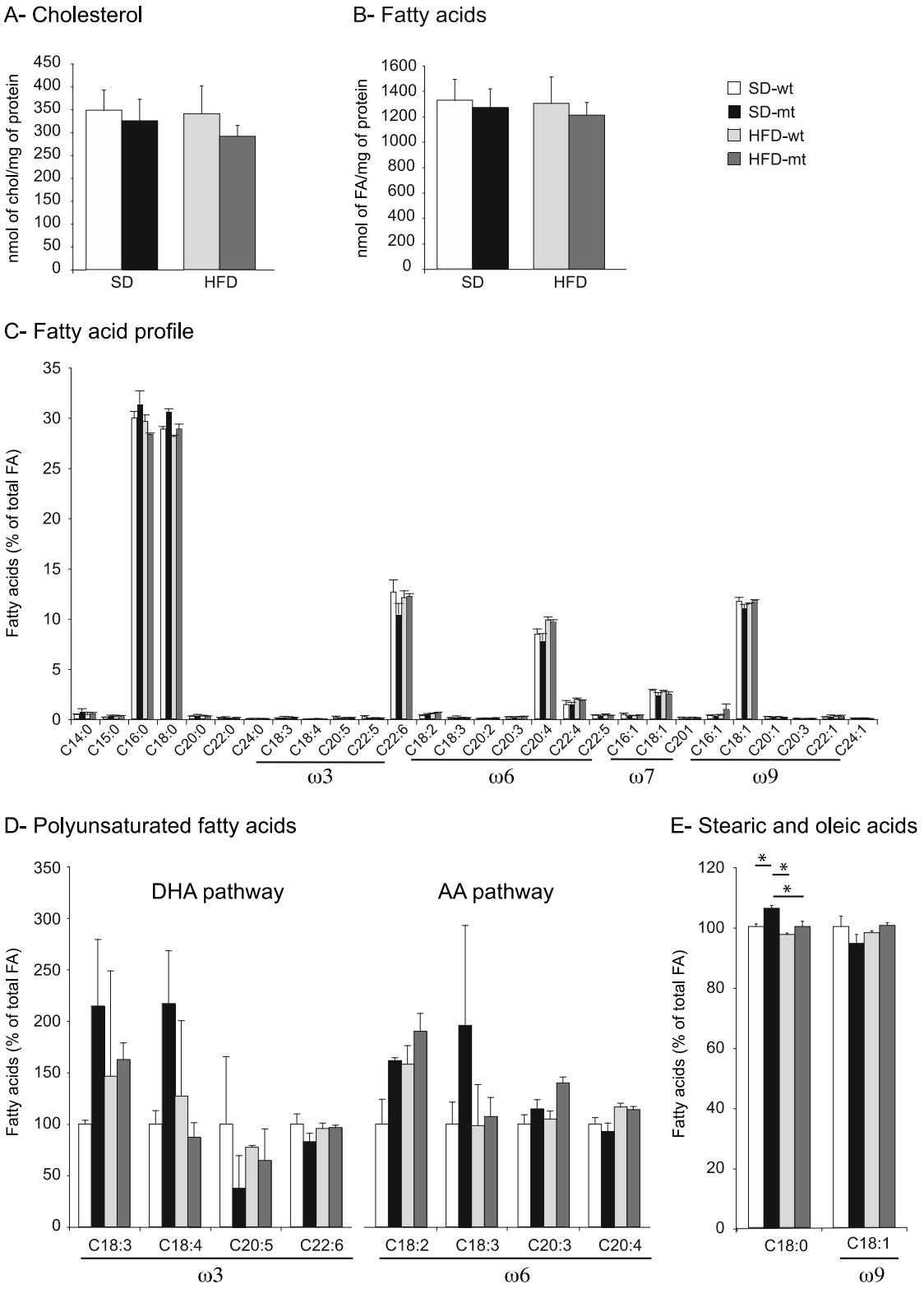

Figure 4. The effect of SCAP deletion and high fat diet on synaptosome lipid composition. Levels of cholesterol (A) and total fatty acids (B) in hippocampal synaptosomes from wild-type (wt) and SCAP mutant (mt) mice fed with standard diet (SD) of high fat diet (HFD), data were normalized to protein levels. C) Fatty acid profile of wt and mt mice fed with SD or HFD, with values given as a percentage of total fatty acids. *(continued on the next page)*

Discussion

Deletion of SCAP in astrocytes versus neurons of the hippocampus

As discussed in the previous chapters, the primary target of cre-mediated recombination driven by the hGFAP promoter is astroglia (Bajenaru et al. 2002), next to some granular neurons in the hippocampus (Fraser et al. 2004) and a small population of oligodendrocytes (Bottelbergs et al. 2010). This is notably very different from the extensively characterized hGFAP-Cre mouse that is used to target radial glia (Malatesta et al. 2003). Thus, the most affected population of cells in our model is likely astroglia. It is of interest to note that neurons have much lower levels of lipid metabolism (Vance et al. 1994; Nieweg et al. 2009; Saito et al. 2009; Pfrieger and Ungerer 2011) than astrocytes, the latter of which are the main contributors of lipids to neurons. Therefore it is most likely that SCAP deletion in astrocytes is the main cause of the observed changes in the hippocampus.

Altered lipid synthesis may underlie impaired hippocampal LTP in SCAP mutants

Our observation that SCAP mutant animals have impaired hippocampal LTP has several plausible explanations. First, altered lipid metabolism in SCAP mutants may involve changes in membrane fluidity, which may affect synaptic vesicle exocytosis (Linetti et al. 2010) and function of membrane-associated proteins, notably neurotransmitter receptors and ion channels (Piomelli et al. 2007). Both, cholesterol and fatty acids are known to affect membrane fluidity. We did not detect notable changes in cholesterol levels in synaptosome membranes of SCAP mutant mice, whereas total brain levels of cholesterol were previously found reduced in these animals (Camargo et al. 2012). However, we found elevated levels of stearic acid in synaptosomes, a saturated fatty acid present at high levels in the synaptic membrane where it is found as a moiety of prominent phospholipids, *e. g.*,, PIP2 (Rodgers and Theibert 2002).

D) Omega-3 (ω3) and Omega-6 (ω6) synthetic pathway leading to the formation of DHA and AA, respectively, for wt and mt fed with SD or HFD. E) Stearic and oleic acid levels. C, D and E, values are given as percentage of total fatty acid and normalized to levels of wild-type animals on SD. Data are presented as means ± SEM. (ANOVA and Tukey post hoc-test *= $p < 0.05$; n=3, *shows difference between wild-types to wild-types in standard diet).

Saturated fatty acid moieties in phospholipids, such as stearic acid, are found in lipid rafts (Maeda et al. 2007) and their abnormal accumulation in SCAP mutants may impair the fluidity properties of the synaptic membrane. Moreover, lipid-mediated signalling may be affected. PIP2 is an intracellular second messenger that upon cleavage by phospholipase C (*e. g.*,, via activation of metabotropic glutamate receptors), generates the second messengers inositol-3-phosphate (which releases Ca^{2+} from the endoplasmic reticulum), and diacyl-glycerol, (which activates multiple effector proteins among which protein kinases C and D) (Piomelli et al. 2007). One might speculate that the observed elevated levels of stearic acid in synaptic membranes of SCAP mutants changes the levels or function of PIP2 causing defective responses to synaptic input that involves PIP2, including signalling of metabotropic glutamate receptors.

Stearic acid is desaturated into oleic acid by stearoyl coenzyme desaturase (SCD), which is a transcriptional target of SREBP1c. We previously found that SCD1 transcripts are reduced in SCAP mutants (Camargo et al. 2012). This suggests that the elevated concentration of stearic acid at the synapse emerges from the impaired conversion to oleic acid. The observation that oleic acid levels are despite this not reduced may be explained by uptake from a different source than astrocytes, most likely from the circulation. Similarly, transformation of EFA into PUFA is dependent on SREBPs, and although the levels of DHA and AA are not changed, we found the accumulation of intermediates in their synthesis. It should be noted that, contrary to the levels of stearic acid, the levels of the EFA C18:3 and C18:4 in the synapse are very low, making it unlikely that their increase interferes with synaptic function.

Finally, the reduced total brain cholesterol levels in SCAP mice that we reported previously (Camargo et al. 2012), might affect synaptic transmission as a consequence of the reduced secreted cholesterol and cholesterol-derived signalling molecules (*e. g.*, neurosteroids) from astrocytes to neurons. Importantly, cholesterol is one of the major astrocyte-derived factors that induces synaptogenesis and enhances synaptic transmission, at least *in vitro* (Mauch et al. 2001; Pfrieger 2009). In the same way, oleic acid signalling from astrocytes to neurons may be compromised in SCAP mutants since the oleic acid level is not reduced in synaptosomes, but it is in the total brain. During development, astrocyte-derived oleic acid acts as a neurotrophic factor (Medina and Tabernero 2002; Rodriguez-Rodriguez et al. 2004; Polo-Hernandez et al.

2010). Moreover, oleic acid enhances NMDA-induced responses in pyramidal neurons of rat cerebral cortex (Nishikawa, 1994) and may have similar effects in neurons in the hippocampus.

Thus, SCAP deletion affects hippocampal function by altering lipid composition of the synapse and/or by compromised lipid modulation of synaptic function by astrocytes. In this study we were able to identify only small changes in specific lipids of synaptosomes, which could be due to the small samples-size. A more comprehensive elucidation of lipid composition of both hippocampal astrocyte and synapse membranes, together with a larger sample size, will be required to obtain insight into this. This analysis should be combined with histological analysis of dendrites and synapses to determine whether a change in the morphology and number of synapses underlies the changes in hippocampus LTP in SCAP mutant mice.

SCAP deletion in astrocytes and a subset of granular neurons in the hippocampus leads to impaired contextual fear conditioning

We found that the performance of SCAP mutant mice in a contextual fear-conditioning paradigm was impaired. The hippocampus processes various properties of contextual experiences, i.e., content and strength, and is known to be crucial for fear memory formation (Mamiya et al. 2009). We used the contextual fear-conditioning paradigm to test hippocampal function in SCAP mutant mice and showed that fear memory was intact after 24 hours. Fear memory was impaired when contextual learning was more challenging, i.e. when a slightly different environment was presented, 6 hours after the shock delivery.

What underlies the impaired contextual fear memory in SCAP mutant mice is not clear and there might be several explanations. We found that SCAP mutants did not associate the shock with a different context, whereas wild-types did. There is now considerable evidence that the acquisition of conditional fear responses can be substantially attributed to forms of LTP at synapses of cortical, thalamic, and hippocampal afferents on cells of the baso-lateral region of the amygdala (Blair and al. 2001). The observed reduction in hippocampal LTP in SCAP mutants is likely to underlie the compromised contextual learning in these animals. Mainly two types of ionotropic glutamate receptors mediate the formation of LTP: the N-methyl-D-aspartate (NMDA) receptors and the amino-3-hydroxy-5-methylisoxazole-4-propionic

acid (AMPA) receptors (Grosshans et al. 2002; Goebel et al. 2005). Interestingly, Tayler et al., found that NMDAR-independent learning only occurred in contexts that were similar to a previously experienced environment (Tayler et al. 2011). When contexts were distinct, NMDAR activation was required for learning. Therefore, our observation that SCAP mutant are only compromised in contextual learning when the environmental context is slightly different, indicates a role for compromised NMDAR activation in this. Whether perturbed NMDAR function indeed underlies impaired LTP and compromised learning in SCAP mutants and how lipid metabolism affects NMDA signalling remains to be determined.

We only observed a difference in the fear response between wild-types and mutants at 6h, but not at 24h after the shock delivery. It is known that short-term memory formation is independent of protein synthesis, unlike long-term memory, which involves gene expression and synthesis of synaptic plasticity related proteins (Impey et al. 1998; Eroglu et al. 2003; Richter and Klann 2009). Short-term memory is confined to the first 6 hours after exposure to a new experience. After these 6 hours the process of long-term memory formation is fully taking place. In our mice, we saw that at 6 hours wild-type mice exhibit a strong fear response whereas mutant mice did not. We might speculate that the mechanism underlying short-term memory in SCAP mutants is impaired and that the formation of the fear memory requires longer time in mutant brains. Measuring fear memory formation at different time-points after training, in similar and different context, and in combination with parallel monitoring of the expression of plasticity related genes might be necessary to understand the mechanisms by which SCAP deletion affects memory.

Altered astrocyte lipid metabolism in brain diseases

An essential role of lipids in synaptic development and function is evident from lipid metabolism disorders in which synaptic dysfunctions and mental retardation are found. For instance, subjects with Smith-Lemli-Opitz (SLOS), one of the most common sterol-associated disorders, have cognitive deficits, and transgenic mouse models for this disease have reduced spine numbers which are associated to cognitive deficits (Jiang et al. 2012). Interestingly, for a number of lipid metabolism disorders that are associated with synaptic dysfunction, a link with compromised astrocyte metabolism has been reported. 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 et al. 1999). In addition, recent studies have shown a strong connection between lipid metabolism, ApoE and the neurodegenerative loss of synaptic plasticity in Alzheimer's disease (Poirier 1996; Poirier 2003; Koffie et al. 2012). The lipids shown to be involved include cholesterol (Poirier 2003) and PUFAs (Calon et al. 2005; Calon 2011). For instance, it has recently been shown that cholesterol binds to the c-terminal domain of beta amyloid providing insight into how elevated levels of neuronal cholesterol increases amyloidogenesis (Barrett et al. 2012). Intriguingly, it was found that the risk of Alzheimer's disease is lower in humans carrying a specific polymorphism in SREBP-1a (Spell et al. 2004). Finally, for Huntington's disease, it was demonstrated that expression of the mutant Huntingtin protein in astrocytes contributes to neuronal damage (Shin et al. 2005), whereas others have demonstrated that mutant Huntingtin leads to reduced SREBP maturation and consequent reduced cholesterol synthesis in these cells (Valenza et al. 2005).

The use of dietary lipids in lipid metabolism-related diseases has got attention for a long time. The need of dietary essential fatty acids for proper development and function of the brain is well-established (Ikemoto et al. 2000) and the effect of dietary fatty acids on this has been studied and tested under different conditions (Yoshida et al. 1998; Okuyama et al. 2007). Whereas brain cholesterol metabolism is thought to be independent of dietary intake or peripheral cholesterol synthesis, there is increasing interest in the use of diets containing cholesterol, as the shielding of the brain from cholesterol seems to be less strict that previously thought (Karasinska et al. 2009; Camargo et al. 2012; Saher et al. 2012). We previously found that the high fat diet, which is rich in cholesterol and mono-unsaturated fatty acids, improved survival and motor dysfunctions in SCAP mutants (Camargo et al. 2012). Here we show that the HFD may also improve LTP in SCAP mutants. It should be noted that HFD also decreased LTP induction in wild-types, which is in line with a deleterious effect of cholesterol on spatial learning that has been reported before (Dufour et al. 2006). We observed that the HFD is unhealthy when given to adult mice for more than 3 months (Camargo et al., 2012), indicating that the positive effects of the HFD on brain function of SCAP mutant mice may be on the long term counteracted by its negative effects. Therefore, we conclude that a fat enriched diet may have positive effects on brain function when lipid metabolism in the brain is perturbed, and that optimization

of this diet should be considered to increase its efficiency. These efforts may be of great advantage in the treatment of cognitive deficits linked to reduced lipid synthesis, such as in SLOS.

Taken together, we showed that SCAP deletion in astrocytes, and possibly in a subset of granular neurons, impaired hippocampal LTP and contextual memory. Furthermore, we showed that a fat-enriched diet normalized altered lipid levels in synaptosomes and improved compromised LTP in SCAP mutant animals. These results have important implications for our understanding of the consequences of disrupted lipid metabolism in the hippocampus and may contribute to elucidate the role of astrocytes in synaptic function. A detailed study on changes in the number of synapses and dendrites, as well as function of specific receptors in the hippocampus after SCAP deletion in GFAP expressing cells will be necessary to understand the cause of impaired LTP and cognitive function disturbance observed in our model and the positive effect of lipid supplementation on this.

Materials and methods

Animals

All experimental procedures were approved by the local animal research committee and complied with the European Council Directive (86/609/EEC). SCAP-floxed mice were from the Jackson Laboratory and have been described (Matsuda et al. 2001). The hGFAP-Cre-IRES-LacZ transgenic mice have been described (Bajenaru et al. 2002) and are referred to as 'GFAP-cre'. Both mouse lines were maintained on a C57Bl6 background. In subsequent generations, we obtained mice with a genotype SCAPloxP/loxP, which are referred to as 'SCAP mutants' (Camargo et al. 2012). Littermates with genotypes SCAPloxP/loxP, SCAPlox/wt or wild-type are referred to as wild-types. Unless indicated otherwise, mice were housed with their littermates of the same gender in Macrolon cages on sawdust bedding, after weaning (three weeks after birth), for the purpose of animal welfare. Food (Harlan Teklad) and water were provided *ad libitum*. Housing was controlled for temperature, humidity and light-dark cycle (7 AM lights on, 7 PM lights off).

Diets

Fat-enriched diets were provided as described in (Camargo et al. 2012), in short:

Pregnant mice, on day 14 of gestation, received either standard diet (TD.02016, Teklad diets, Harlan Laboratories, Madison, WI, USA), or a high fat diet containing 60% fat calories diet (TD.09167). Diets were given to the pregnant mice, during lactation and after weaning.

Slice electrophysiology

Hippocampal coronal slices (400 μm thickness) were prepared from 2.5 month-old SCAP mutants (n=18) or littermate controls (n=15). For the diet experiment, animals were 2.5 to 3 month-old with the following groups: SCAP mutants on standard diet (n=15) or high fat diet (n=16), and controls on standard diet (n=20) or high fat diet (n=21). Slices were cut in ice-cold slicing artificial cerebro-spinal fluid (ACSF) containing the following (in mM): 125 NaCl, 3 KCl, 1.25 NaH2PO4, 26 NaHCO3, 10 glucose, 3 MgSO4, and 1 CaCl2 (0.300 mOsm), and carboxygenated with 95%O2/ 5%CO2. Slices were allowed to equilibrate for 1 h at room temperature in modified carboxygenated ACSF containing the following (in mM): 125 NaCl, 3 KCl, 1.25 NaH2PO4, 26 NaHCO3, 10 glucose, 1 MgSO4, 3 CaCl2, and 0.01 glycine (300 mOsm). Slices were put onto a 64-channel multielectrode recording probe (MED64; Alpha MED Sciences) with the electrodes covering CA1, and superfused with 4 ml/min carboxygenated ACSF at 32°C. Inputs from CA3 region were stimulated with a bipolar extracellular electrode placed in stratum radiatum (0.15 ms; current was adjusted per experiment to evoke half-maximum response), and synaptic response was quantified as the maximum amplitude of the field EPSP (fEPSP) response. After 20 min baseline recording at 0.05 Hz, long-term potentiation (LTP) was induced by theta burst stimulation (TBS; eight trains of four pulses at 100 Hz, delivered at 200 ms intervals). TBS was performed three times at 10 s intervals. After LTP induction, fEPSPs were recorded for 40 min at 0.05 Hz.

Fear conditioning

The fear-conditioning test was carried out in a fear conditioning system (TSE system) and consisted of 3 phases (with some modifications for the diet experiments, see below): 1) training phase in Box1, 2) testing phase (+ 6 hours) in Box2, and 3) testing phase (+24 hours) in Box1. The training chamber (Box1) consisted of a rectangular plexiglas cage (36 × 21 × 20 cm) within a constantly illuminated fear conditioning

box made of dark grey acrylic plastic. The conditioning box was provided with a constant auditory white noise at 68 dB. Mice were taken individually in their home cages to a dimly lit room, where they were immediately transferred to the test chamber and allowed to explore it for a period of 3 min. This was followed by a foot shock (0.7mA), via a shock grid floor for a period of 2s. After an additional 30s, the animals were removed from the test chamber, and removed from the testing room while in their home cage. After 6 hours the mice were placed in a different cage (Box2), which was no longer placed in the illuminated fear conditioning dark acrylic plastic box; instead the Box2 was placed on top of a vibration free table. This box had a different illumination and was cleaned with acetate, unlike Box1 that was cleaned with 70% ethanol. Box2 did however contain the grid floor, which was used to deliver the foot shock in Box1. The mice were allowed to explore this box for 3 minutes. No foot shock was delivered and the mice were placed back in their home cage and removed from the test room. The same group of animals was tested 24 hours after the shock by placing the animals in the exactly same conditions as in which the shock was delivered (Box1), however no foot shock was delivered and the animals were allowed to explore for 3 minutes.

For the diet experiments, mutant and wild-type animals were fed with a high fat diet, as described above. The protocol used was the same, except that the Box2 was omitted. The software integrated to the TSE system determined inactivity and exploration automatically.

Synaptosome lipid composition

For synaptosome isolation, one hippocampus per animal (n=3, for wild-types and mutants on either standard diet or high fat diet) were homogenized in ice-cold 0.32 M sucrose buffer at pH 7.4 and centrifuged at 1000 x g for 10 min. Supernatant was loaded on top of a discontinuous sucrose gradient consisting of 0.85 M and 1.2 M sucrose. After centrifugation for 2 h at 110.000 x *g*, the synaptosomal fraction at the interface of 0.85 M and 1.2 M sucrose was collected, rediluted in 0.32 M sucrose buffer and the synaptosome fraction was resuspended in 500 µL of 500 mM Hepes after centrifugation. Protein concentrations were determined using a Bradford assay (Bio-Rad). The relative abundances of lipid species in the sample-extracts were determined, using HPLC-MS/MS. The liquid-chromatographic separation was

achieved on a modular HPLC system (Surveyor; Thermo Finnigan, San Jose, CA, USA) consisting of a cooled autosampler (T=12 °C), a low-flow quaternary MS pump and analytical HPLC column: 2.1×250 mm silica column, 5 μm particle diameter (Merck, Darmstadt, Germany). Samples were eluted with a flow rate of 300 µl/min and a programmed linear gradient between solution B (chloroform-methanol, 97:3, v/v) and solution A (methanol-water, 85:15, v/v); A and B contained 0.1 ml and 0.01 ml of 25% (v/v) aqueous ammonia per liter of eluent, respectively: the gradient was: T = 0 -10 min: 20% A to 100% A; T = 10-12 min, 100% A; T= 12-12.1 min: 100% A to 0% A; and T= 12.1–17 min, equilibration with 0% A. Total run-time, including the equilibration, was 17 min. A splitter between the HPLC and MS was used for the introduction of the eluent in the MS by 75 μl/min.

MS/MS analyses were performed on a TSQ Quantum II (Thermo Finnigan Corporation, San Jose, CA, USA) operated alternating in the negative- and positive ion electrospray ionization (ESI) mode in consecutive runs. The SID was set at 10 V; spray voltage was 3600 V and the capillary temperature was 300 °C. In the MS/MS experiments Ar was used as collision gas at a pressure of 0.5 mtorr; collision energy ranged between 20-40 eV for the different optimized transitions. In the negative mode mass spectra were obtained by continuous scanning between m/z 400 - m/z 1000 (2s/scan). In the positive mode characteristic constant neutral loss (CNL) or parent (P) scans were used to selectively detect specific phospholipids in their corresponding retention time windows.

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References

Allen JA, Halverson-Tamboli RA & Rasenick MM (2007) Lipid raft microdomains and neurotransmitter signalling. *Nat Rev Neurosci* **8**, 128-140.

Bajenaru, M.L., Zhu, Y., Hedrick, N.M., Donahoe, J., Parada, L.F., and Gutmann, D.H. 2002. Astrocyte-specific inactivation of the neurofibromatosis 1 gene (NF1) is insufficient for astrocytoma formation. *Mol Cell Biol* **22**(14): 5100-5113.

Barrett, P.J., Song, Y., Van Horn, W.D., Hustedt, E.J., Schafer, J.M., Hadziselimovic, A., Beel, A.J., and Sanders, C.R. 2012. The amyloid precursor protein has a flexible transmembrane domain and binds cholesterol. *Science* **336**(6085): 1168-1171.

Belelli, D. and Lambert, J.J. 2005. Neurosteroids: endogenous regulators of the GABA(A) receptor. *Nat Rev Neurosci* **6**(7): 565-575.

Bilunas, M.H. and Moore, S.A. 2002. Cyclooxygenase-2 expression and activity are induced by amyloid-beta 1-42 in brain derived cells. *Adv Exp Med Biol* **507**: 93-99.

Bottelbergs, A., Verheijden, S., Hulshagen, L., Gutmann, D.H., Goebbels, S., Nave, K.A., Kassmann, C., and Baes, M. 2010. Axonal integrity in the absence of functional peroxisomes from projection neurons and astrocytes. *Glia* **58**(13): 1532-1543.

Calon, F. 2011. Omega-3 polyunsaturated fatty acids in Alzheimer's disease: key questions and partial answers. *Curr Alzheimer Res* **8**(5): 470-478.

Calon, F., Lim, G.P., Morihara, T., Yang, F., Ubeda, O., Salem, N., Jr., Frautschy, S.A., and Cole, G.M. 2005. Dietary n-3 polyunsaturated fatty acid depletion activates caspases and decreases NMDA receptors in the brain of a transgenic mouse model of Alzheimer's disease. *Eur J Neurosci* **22**(3): 617-626.

Camargo, N., Brouwers, J., Loos, M., Gutmann, D., Smit, A., and Verheijen, M. 2012. High-Fat Diet Ameliorates Neurological Deficits Caused by Defective Astrocyte Lipid Metabolism. *FASEB J*. Epub ahead of print.

Dufour, F., Liu, Q.Y., Gusev, P., Alkon, D., and Atzori, M. 2006. Cholesterol-enriched diet affects spatial learning and synaptic function in hippocampal synapses. *Brain Res* **1103**(1): 88-98.

Eroglu, C., Brugger, B., Wieland, F., and Sinning, I. 2003. Glutamate-binding affinity of Drosophila metabotropic glutamate receptor is modulated by association with lipid rafts. *Proc Natl Acad Sci U S A* **100**(18): 10219-10224.

Fraser, M.M., Zhu, X., Kwon, C.H., Uhlmann, E.J., Gutmann, D.H., and Baker, S.J. 2004. Pten loss causes hypertrophy and increased proliferation of astrocytes in vivo. *Cancer Res* **64**(21): 7773-7779.

Frye, C.A. and Sturgis, J.D. 1995. Neurosteroids affect spatial/reference, working, and long-term memory of female rats. *Neurobiol Learn Mem* **64**(1): 83-96.

Hering, H., Lin, C.C., and Sheng, M. 2003. Lipid rafts in the maintenance of synapses, dendritic spines, and surface AMPA receptor stability. *J Neurosci* **23**(8): 3262-3271.

Ikemoto, A., Nitta, A., Furukawa, S., Ohishi, M., Nakamura, A., Fujii, Y., and Okuyama, H. 2000. Dietary n-3 fatty acid deficiency decreases nerve growth factor content in rat hippocampus. *Neurosci Lett* **285**(2): 99-102.

Impey, S., Smith, D.M., Obrietan, K., Donahue, R., Wade, C., and Storm, D.R. 1998. Stimulation of cAMP response element (CRE)-mediated transcription during contextual learning. *Nat Neurosci* **1**(7): 595-601.

Jang, D.J., Park, S.W., and Kaang, B.K. 2009. The role of lipid binding for the targeting of synaptic proteins into synaptic vesicles. *BMB Rep* **42**(1): 1-5.

Jiang, X.S., Wassif, C.A., Backlund, P.S., Song, L., Holtzclaw, L.A., Li, Z., Yergey, A.L., and Porter, F.D. 2012. Activation of Rho GTPases in Smith-Lemli-Opitz syndrome: pathophysiological and clinical implications. *Hum Mol Genet* **19**(7): 1347-1357.

Karasinska, J.M., Rinninger, F., Lutjohann, D., Ruddle, P., Franciosi, S., Kruit, J.K., Singaraja, R.R., Hirsch-Reinshagen, V., Fan, J., Brunham, L.R., Bissada, N., Ramakrishnan, R., Wellington, C.L., Parks, J.S., and Hayden, M.R. 2009. Specific loss of brain ABCA1 increases brain cholesterol uptake and influences neuronal structure and function. *J Neurosci* **29**(11): 3579-3589.

Koffie, R.M., Hashimoto, T., Tai, H.C., Kay, K.R., Serrano-Pozo, A., Joyner, D., Hou, S., Kopeikina, K.J., Frosch, M.P., Lee, V.M., Holtzman, D.M., Hyman, B.T., and Spires-Jones, T.L. 2012. Apolipoprotein E4 effects in Alzheimer's disease are mediated by synaptotoxic oligomeric amyloid-beta. *Brain* **135**(Pt 7): 2155-2168.

Kotti, T., Head, D.D., McKenna, C.E., and Russell, D.W. 2008. Biphasic requirement for geranylgeraniol in hippocampal long-term potentiation. *Proc Natl Acad Sci U S A* **105**(32): 11394-11399.

Kotti, T.J., Ramirez, D.M., Pfeiffer, B.E., Huber, K.M., and Russell, D.W. 2006. Brain cholesterol turnover required for geranylgeraniol production and learning in mice. *Proc Natl Acad Sci U S A* **103**(10): 3869-3874.

Malatesta, P., Hack, M.A., Hartfuss, E., Kettenmann, H., Klinkert, W., Kirchhoff, F., and Gotz, M. 2003. Neuronal or glial progeny: regional differences in radial glia fate. *Neuron* **37**(5): 751-764.

Mamiya, N., Fukushima, H., Suzuki, A., Matsuyama, Z., Homma, S., Frankland, P.W., and Kida, S. 2009. Brain region-specific gene expression activation required for reconsolidation and extinction of contextual fear memory. *J Neurosci* **29**(2): 402-413.

Matsuda, M., Korn, B.S., Hammer, R.E., Moon, Y.A., Komuro, R., Horton, J.D., Goldstein, J.L., Brown, M.S., and Shimomura, I. 2001. SREBP cleavage-activating protein (SCAP) is required for increased lipid synthesis in liver induced by cholesterol deprivation and insulin elevation. *Genes Dev* **15**(10): 1206-1216.

Mauch, D.H., Nagler, K., Schumacher, S., Goritz, C., Muller, E.C., Otto, A., and Pfrieger, F.W. 2001. CNS synaptogenesis promoted by glia-derived cholesterol. *Science* **294**(5545): 1354-1357.

Medina, J.M. and Tabernero, A. 2002. Astrocyte-synthesized oleic acid behaves as a neurotrophic factor for neurons. *J Physiol Paris* **96**(3-4): 265-271.

Nagler, K., Mauch, D.H., and Pfrieger, F.W. 2001. Glia-derived signals induce synapse formation in neurones of the rat central nervous system. *J Physiol* **533**(Pt 3): 665-679.

Nieweg, K., Schaller, H., and Pfrieger, F.W. 2009. Marked differences in cholesterol synthesis between neurons and glial cells from postnatal rats. *Journal of neurochemistry* **109**(1): 125-134.

Okuyama, H., Yamada, K., Miyazawa, D., Yasui, Y., and Ohara, N. 2007. Dietary lipids impacts on healthy ageing. *Lipids* **42**(9): 821-825.

Pfrieger, F.W. 2009. Roles of glial cells in synapse development. *Cell Mol Life Sci* **66**(13): 2037-2047.

Pfrieger, F.W. and Ungerer, N. 2011. Cholesterol metabolism in neurons and astrocytes. *Progress in lipid research* **50**(4): 357-371.

Piomelli, D. 1993. Arachidonic acid in cell signaling. *Curr Opin Cell Biol* **5**(2): 274-280.

Piomelli, D., Astarita, G., and Rapaka, R. 2007. A neuroscientist's guide to lipidomics. *Nat Rev Neurosci* **8**(10): 743-754.

Poirier, J. 1996. Apolipoprotein E in the brain and its role in Alzheimer's disease. *J Psychiatry Neurosci* **21**(2): 128-134.

Poirier, J. 2003. Apolipoprotein E and cholesterol metabolism in the pathogenesis and treatment of Alzheimer's disease. *Trends Mol Med* **9**(3): 94-101.

Polo-Hernandez, E., De Castro, F., Garcia-Garcia, A.G., Tabernero, A., and Medina, J.M. Oleic acid synthesized in the periventricular zone promotes axonogenesis in the striatum during brain development. *J Neurochem* **114**(6): 1756-1766.

Richter, J.D. and Klann, E. 2009. Making synaptic plasticity and memory last: mechanisms of translational regulation. *Genes Dev* **23**(1): 1-11.

Rodgers, E.E. and Theibert, A.B. 2002. Functions of PI 3-kinase in development of the nervous system. *Int J Dev Neurosci* **20**(3-5): 187-197.

Rodriguez-Rodriguez, R.A., Tabernero, A., Velasco, A., Lavado, E.M., and Medina, J.M. 2004. The neurotrophic effect of oleic acid includes dendritic differentiation and the expression of the neuronal basic helix-loop-helix transcription factor NeuroD2. *J Neurochem* **88**(5): 1041-1051.

Saher, G., Rudolphi, F., Corthals, K., Ruhwedel, T., Schmidt, K.F., Lowel, S., Dibaj, P., Barrette, B., Mobius, W., and Nave, K.A. 2012. Therapy of Pelizaeus-Merzbacher disease in mice by feeding a cholesterol-enriched diet. *Nat Med*.

Saito, K., Dubreuil, V., Arai, Y., Wilsch-Brauninger, M., Schwudke, D., Saher, G., Miyata, T., Breier, G., Thiele, C., Shevchenko, A., Nave, K.A., and Huttner, W.B. 2009. Ablation of cholesterol biosynthesis in neural stem cells increases their VEGF expression and angiogenesis but causes neuron apoptosis. *Proc Natl Acad Sci U S A* **106**(20): 8350-8355.

Slezak, M. and Pfrieger, F.W. 2003. New roles for astrocytes: regulation of CNS synaptogenesis. *Trends Neurosci* **26**(10): 531-535.

Tabernero, A., Lavado, E.M., Granda, B., Velasco, A., and Medina, J.M. 2001. Neuronal differentiation is triggered by oleic acid synthesized and released by astrocytes. *J Neurochem* **79**(3): 606-616.

Tabernero, A., Velasco, A., Granda, B., Lavado, E.M., and Medina, J.M. 2002. Transcytosis of albumin in astrocytes activates the sterol regulatory element-binding protein-1, which promotes the synthesis of the neurotrophic factor oleic acid. *J Biol Chem* **277**(6): 4240-4246.

Tayler, K.K., Lowry, E., Tanaka, K., Levy, B., Reijmers, L., Mayford, M., and Wiltgen, B.J. 2011. Characterization of NMDAR-Independent Learning in the Hippocampus. *Front Behav Neurosci* **5**: 28.

Valianpour, F., Wanders, R.J., Barth, P.G., Overmars, H., and van Gennip, A.H. 2002. Quantitative and compositional study of cardiolipin in platelets by electrospray ionization mass spectrometry: application for the identification of Barth syndrome patients. *Clin Chem* **48**(9): 1390-1397.

Vance, J.E., Pan, D., Campenot, R.B., Bussiere, M., and Vance, D.E. 1994. Evidence that the major membrane lipids, except cholesterol, are made in axons of cultured rat sympathetic neurons. *J Neurochem* **62**(1): 329-337.

Vreugdenhil, M., Bruehl, C., Voskuyl, R.A., Kang, J.X., Leaf, A., and Wadman, W.J. 1996. Polyunsaturated fatty acids modulate sodium and calcium currents in CA1 neurons. *Proc Natl Acad Sci U S A* **93**(22): 12559-12563.

Yoshida, S., Sato, A., and Okuyama, H. 1998. Pathophysiological effects of dietary essential fatty acid balance on neural systems. *Jpn J Pharmacol* **77**(1): 11-22.