Saturated and unsaturated fat diets impair hippocampal glutamatergic transmission in adolescent mice

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Keywords: 
SOLF
UOLF
LTP
LTD
Synaptic plasticity
Memory

ARTICLE INFO

ABSTRACT

Consumption of high-fat diets (HFD) has been associated with neuronal plasticity deficits and cognitive disorders linked to the alteration of glutamatergic disorders in the hippocampus. As young individuals are especially vulnerable to the effects of nutrients and xenobiotics on cognition, we studied the effect of chronic consumption of saturated (SOLF) and unsaturated oil-enriched foods (UOLF) on: i) spatial memory; ii) hippocampal synaptic transmission and plasticity; and iii) gene expression of glutamatergic receptors and hormone receptors in the hippocampus of adolescent and adult mice. Our results show that both SOLF and UOLF impair spatial short-term memory. Accordingly, hippocampal synaptic plasticity mechanisms underlying memory, and gene expression of NMDA receptor subunits are modulated by both diets. On the other hand, PPARγ gene expression is specifically down-regulated in adolescent SOLF individuals and up-regulated in adult UOLF mice.

1. Introduction

Consumption of high-fat diets (HFD) has been associated with both neuronal plasticity deterioration and cognitive deficits linked to the impairment of glutamatergic neurotransmission within the hippocampus (HIP) (Del Olmo and Ruiz-Gayo, 2018). These deficiencies are particularly intense in individuals that start to consume this type of diets during pre-adulthood, as demonstrated by studies comparing the effect of HFD in young and adult mice and showing that HFD worsen both relational and spatial memory capacities, specifically in animals that consume these diets during the juvenile period (Boitard et al., 2012; Del Rio et al., 2016; Kaczmarczyk et al., 2013; Valladolid-Acebes et al., 2013, 2011). Memory decline induced by HFD overlaps with the impairment of synaptic efficacy and blunting of NMDA-induced long-term depression (LTD) within the HIP (Valladolid-Acebes et al., 2012). The deterioration of synaptic plasticity triggered by HFD occurs concomitantly with changes in glutamatergic neurotransmission, as assessed in ex vivo neurochemical studies showing that HFD consumption decreases the efficiency of glutamate (GLU) up-take and evokes an adaptive up-regulation of glial GLU transporters (GLT-1 and GLAST) as well as a down-regulation of glutamine synthase within the HIP (Valladolid-Acebes et al., 2012).

The above-mentioned changes are apparently not due to the resulting obesity as they are detected before obesity is established and have been linked to brain insulin resistance (Beilharz et al., 2015; Grillo et al., 2015; Vinuesa et al., 2016). Other studies have reported that deficient leptin receptor (Lepr) signalling triggered by HFD intake might also account for HIP function impairment (Cordner and Tamashiro, 2015; Dodds et al., 2011; Hwang et al., 2010; Mainardi et al., 2017; McGregor and Harvey, 2018; Valladolid-Acebes et al., 2012). It is suggested that either specific fatty acids (FAs) or a certain ratio of saturated vs. unsaturated FAs yielded by HFD consumption could account for HIP

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolpropionic acid; AMPAR, AMPA receptor; BST, Basal synaptic transmission; BW, Body weight; FA, Fatty acid; fEPSP, Field excitatory postsynaptic potentials; GLU, Glutamate; HFD, High-fat diet; HIP, Hippocampus; Lepr, Leptin receptor; NMDA, N-methyl-D aspartate; NMDAR, NMDA receptor; SOLF, Saturated oil-enriched food; UOLF, Unsaturated oil-enriched food.

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https://doi.org/10.1016/j.psyneuen.2021.105429
Received 19 June 2020; Received in revised form 10 September 2021; Accepted 20 September 2021
Available online 24 September 2021
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dysfunction independently of other factors (Del Olmo and Ruiz-Gayo, 2018). Moreover, although no previous study has demonstrated that saturated FA intake is an independent risk factor for memory and mood decline, population studies carried out in children/adolescents point to a negative correlation between HIP-dependent relational memory and saturated fat intake, independently of metabolic factors (Baym et al., 2014). Furthermore, HIP-dependent memory deficits have been observed in adolescent mice that display HFD-induced obesity, but not systemic insulin resistance (Valladolid-Acebes et al., 2011).

Related to the work from other groups (Spinelli et al., 2017), the present study is based on the hypothesis that regular intake of diets containing elevated amounts of saturated, but not unsaturated FAs is detrimental for HIP mechanisms that underlie learning/memory, particularly in individuals that consume these diets during the adolescence/early juvenile period. To address this issue, diets enriched with either saturated (SOLF, Saturated Oil-enriched Food) or unsaturated fat (UOLF, Unsaturated Oil-enriched Food) were provided for 8 weeks to 5- (adolescent) and 8-weekold (young adult) mice, and their effects on: i) spatial short-term memory; ii) synaptic transmission and plasticity, and iii) gene and protein expression of glutamate, hormone and peroxisome proliferator-activated (PPARs) receptors, were characterized in the HIP.

2. Material and methods

2.1. Diets, animals, and experimental design

The two HFDs used in this study were manufactured in our laboratory as previously described (Plaza et al., 2019) from standard rodent chow (60%; SD, Teklad global 2018, Harlan Laboratories, IN, USA) and 40% of either high-oleic sunflower oil (Unsaturated Oil-enriched Food, UOLF) or palm kernel oil (Saturated Oil-enriched Food, SOLF) (see diets’ composition in Supplementary Material, Table 1).

Study 1

P35 (n = 7 mice/group)  
8-week dietary treatment  
P55 (n = 7 mice/group)  
Adolescent cohort  
Adult cohort  
Weekly monitoring of food intake and body weight  
Plasma biochemistry  
Behavioral test  
PCR  
Western blot

Study 2

P35 (n = 17 mice/group)  
8-week dietary treatment  
Dietary treatment  
SD (Standard diet)  
SOLF († Saturated fat)  
UOLF († Unsaturated fat)  
Adolescent cohort  
Weekly monitoring of food intake and body weight  
Plasma biochemistry  
EPSP measures

Fig. 1. Schematic illustration of the dietary protocol. The research was organized in two studies. Study 1 was carried out both in adolescent and adult animals that started to consume the experimental diets on postnatal days 35 (P35) and 55 (P55), respectively. Study 2 was carried out only in adolescent animals. In both studies food intake and BW were monitored weekly. Animals from Study 1 were tested in the Y-maze at the end of the dietary treatment, then killed and HIP samples were used for WB and RT-PCR. Animals from Study 2 were used in electrophysiological experiments.
monocyte chemoattractant protein-1 (MCP1) were measured in a multiplex immunoassay (Millipore, Billerica, MA) in a Bio-Plex suspension array system 200 (Bio-Rad Laboratories, Hercules, CA, USA). Adiponectin was analysed by ELISA (Abcam, UK). Triglycerides (Spinreact, Spain) and non-esterified FA (NEFA) (Wako Bioproducts, USA) were measured by spectrophotometric methods.

2.3. Evaluation of spatial memory in the Y maze behavioral testing

Spatial short-term working memory was analyzed by recording spontaneous alternation in a black plexiglass Y maze conformed by three identical arms (50 cm long x 19 cm wide) with 35 cm high walls (Contreras et al., 2019). Animals were placed in the testing room, which was rich in visual cues, 60 min before testing for habituation. For testing, mice were placed into one arm, and allowed to explore the maze for 10 min, during which arm entries (all four paws within an arm) and decision time (in the center of the maze) were recorded. A correct alternation was defined as an entry into three different arms (A, B and C) in overlapping successive sequences of 3 arm entries (e.g., a successive alternation was defined as an entry into three different arms (A, B and C) in overlapping successive sequences of 3 arm entries (A, C, B, C, B, A, C, A, B)). The percent alternation score was calculated as \[ \left( \frac{\text{actual alternations}}{\text{possible alternations}} \right) \times 100. \]

2.4. Electrophysiology assays

Briefly, transverse HIP slices (400 µm thick) were prepared by using a manual tissue chopper (Stoelting Tissue Slicer, Illinois) and placed in gassed (95% O2, 5% CO2) ice-cold Krebs–Ringer bicarbonate (KRB) solution containing (mM): 119 NaCl, 2.5 KCl, 1 KH2PO4, 1.3 MgSO4, 2.5 CaCl2, 26.2 NaHCO3 and 11 glucose, in a humidified interface chamber at 20–25°C, as described previously (Del Olmo et al., 2000, 2003). After 2 h incubation, the slices were transferred to the submersion recording chamber, where they were continuously perfused (2 ml/min) with standard KRB solution. Field excitatory postsynaptic potentials (fEPSPs) were evoked by stimulating Schaffer collateral commissural fibers with bipolar electrical pulses (30-70 µA and 100 µs at 0.033 or 0.066 Hz) delivered through bipolar tungsten insulated microelectrodes (0.5 MΩ) and recorded in the CA1 stratum radiatum using tungsten electrodes (1 MΩ). Electrical pulses were generated by a pulse generator Master 8 (AMPI, Israel) and the recording electrode was connected to an AI-402 amplifier (Axon Instruments, USA) connected in turn to a CyberAmp 320 signal conditioner (Axon Instruments, USA). Evoked responses were digitized at 25–50 Hz using a Digidata 1320 A (Axon Instruments, USA) and stored on a Pentium IV IBM-compatible computer using pCLAMP 9.0 software (Axon Instruments, USA). The Schaffer collateral axons contacting the cell population of interest were stimulated every 30 s and after obtaining stable synaptic responses for at least 20 min (baseline period) an experimental protocol was performed. Synaptic plasticity was studied by inducing long-term potentiation (LTP) by four high-frequency stimulation (HFS) trains (100 Hz, 1 s, at test intensity) separated by 20 s. In another set of experiments, slices were perfused with 15 µM NMDA for 6 min to induce NMDA-LTD, following the protocol previously used in our laboratory (Lee et al., 1998; Naranjo et al., 2019; Valladao-Acebes et al., 2012). Synaptic strength was assessed by measuring the initial slope of the fEPSP and the data were normalized with respect to the mean values of the responses of each animal during the 20 min baseline period. A single slice from each individual animal was considered as n = 1. All electrophysiological experiments were carried out at 31–32°C. In some of the experiments and during the baseline period, input/output (I/O) curves were applied to evaluate basal synaptic transmission (BST).

2.5. RT-PCR

Total HIP RNA was extracted by using the Tri-Reagent protocol (Sigma, USA). cDNA was then synthesized from 1 µg total mRNA by using a high-capacity cDNA RT kit (BioRad, CA). Quantitative RT-PCR was performed by using designed primer pairs (Integrated DNA Technologies, USA. Table 2, Supplementary Material). SsoAdvanced Universal SYBR Green Supermix (BioRad, CA) was used for amplification according to the manufacturer’s protocols, in a CFX96 Real Time System (BioRad). Values were normalized to the housekeeping genes Actb and 18s. The ΔΔC(T) method was used to determine relative expression levels. Statistics were performed using ΔΔC(T) values (Livak and Schmittgen, 2001).

2.6. Western-blot assays

HIP were homogenized (2 cycles of 50 Hz 1 min each one; Tissue-Lyser, Qiagen, Spain) in 500 µl ice-cold buffer (0.42 M NaCl, 1 mM Na2PO4, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 20% glycerol, 1 mg/ml aprotinin, 1 mg/ml leupeptin, 1 mg/ml Nα-tosyl-L-lysine chloromethyl ketone hydrochloride, 20 mM NaF, 1 mM trisodium orthovanadate, and 2 mM phenylmethylsulphonyl fluoride in 20 mM HEPES pH=7.9), submitted to three consecutive freezing/thawing (~80°C/37°C) cycles, and centrifuged (10 min, 4°C). Proteins in the supernatant were quantified (Bradford assay) and their final concentration adjusted to 1 µg/µl in Laemli buffer (0.125 mM Tris, pH=6.8 containing 2% SDS, 25% glycerol, 5% β-mercaptoethanol, and 0.01% blue bromophenol). After boiling (5 min), 15 µL samples were loaded in 6% SDS-polyacrylamide gels and submitted to electrophoresis (glycine 0.2 M and SDS 0.1% in TRIS 0.025 M). Proteins were transferred to 0.2 µm nitrocellulose membranes (Bio-Rad, Spain) by using the Trans-Blot Turbo Transfer System and the corresponding Transfer Pack (Bio-Rad, Spain). Membranes were washed (0.1% non-fat dried milk, 0.5% tween 20 in PBS; 2 × 5 min), blocked (5% non-fat dried milk; 0.5% tween-20 in PBS; 1 h), incubated with primary antibodies/antisera (12 h, 4°C), and finally re-incubated with appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies (30 min, 25°C) (Table 3, Supplementary Material). Blots were washed, incubated in chemiluminescence reagents (ECL Prime; GE Healthcare Life Sciences), and the bands detected using the ChemiDoc XRS+ Imaging System (BioRad). To check the uniformity of sample loading, blots were re-incubated with β-actin monoclonal antibody (Affinity Bioreagents, CO).

2.7. Statistical analysis

All data are presented as the means ± S.E.M. Statistical significance was determined by one-way ANOVA (ANOVA-1) followed by Bonferroni’s post hoc test or two-way ANOVA (ANOVA-2) when appropriate. Outliers were identified by using the ROUT method (Q = 1%; GraphPad Prism software).

3. Results

3.1. Spontaneous alternation is modulated by diets enriched either in saturated or in unsaturated fatty acids

Spontaneous alternation performance was tested in the Y maze both in adolescent and adult mice that consumed either SOLF or UOLF. The percentage of spontaneous alternation was analyzed in adolescent and adult cohorts after dietary treatment, as illustrated in Fig. 2. Statistical analysis by ANOVA revealed an effect of dietary treatment (P < 0.05) while no effect of age nor significant interaction dietary treatment x age was observed.

3.2. SOLF and UOLF regulate the expression of both NMDA2A (Grin2A gene) and NMDA2B (Grin2B gene) subunits of the NMDAR in the HIP

Fig. 3A and B show that dietary treatment had no effect on Grin1 and Grin2 gene expression, which suggests that AMPAR subunit levels are not modulated by SOLF/UOLF diets. With respect to the NMDAR subunits (Fig. 3D and E), a down-regulation of both (Grin2A and Grin2B gene...
expression was triggered by the dietary treatment (ANOVA-2; $P < 0.05$ and, $P < 0.05$, respectively). In the case of Grin2A, a significant effect of age ($P < 0.01$) was found, whereas no effect in the interaction was detected. For Grin2B, both age and the interaction age x diet were not significant (Fig. 3B). No effects on Grin1 subunit expression were detected (Fig. 3C).

To assess the effect of SOLF on Grin1, Grin2A and Grin2B expression, their correspondent encoded proteins were quantified by WB in adolescent mice. As illustrated in Fig. 3G-H, SOLF tended to reduce both NMDA2A and NMDA2B immunoreactivity within the HIP although this effect did not reach statistical significance.

### 3.3. Peroxisome proliferator activated receptor gamma (Pparg) was down-regulated by SOLF in the hippocampus of adolescent mice

Gene expression of hormone receptors potentially sensitive to HFDs, such as Ppara and Pparg, insulin (Insr), adiponectin (Adipor1 and Adipor2) and leptin (Lepr) receptors, was measured in the HIP. In the case of Ppara, no effect of the dietary treatment was observed either in adolescent or in adult mice (Fig. 3I). In contrast, Pparg expression was dependent on dietary treatment (ANOVA-2; $P > 0.001$), animal age ($P < 0.001$) and interaction diet x age ($P < 0.001$). Lepr, Adipor and Insr were not affected by the dietary treatment in either adolescent or adult mice (Fig. 3K-N).

### 3.4. SOLF and UOLF differently affected basal synaptic transmission and LTD/LTP in the hippocampus of adolescent mice

Since previous results, obtained by other authors and ourselves (Boitard et al., 2012; Valladolid-Acebes et al., 2011, 2013), indicate a more potent effect of HFD in adolescent mice, electrophysiological studies were carried out only in this group of animals.

As illustrated in Fig. 4A, both diets affected BST. SOLF blunted input/output (I/O) curves (Fig. 4A) indicating a negative impact of this diet on synaptic efficacy in CA1 pyramidal neurons. A similar effect was triggered by UOLF, although in this case the difference did not reach statistical significance.

The effect of both SOLF and UOLF on HIP synaptic plasticity was further characterized by analyzing their effect on LTD and LTP. Regarding LTD, statistical analysis of the recording by ANOVA-2 indicates significant differences in the factors diet ($P < 0.05$), time ($P < 0.001$) and interaction diet x time ($P < 0.001$; post hoc analysis are included in Fig. 4B). As illustrated in Fig. 4B and C, the magnitude of LTD observed 10 min after HFS was significantly attenuated by SOLF (mean ± S.E.M. of fEPSP 10 min after HFS; SD: 148.9 ± 5.0 vs. SOLF, 113.4 ± 2.5). Moreover, SOLF mitigated LTD maintenance 2 h after induction (mean ± S.E.M. of the fEPSP during the last ten minutes of the recording; SD: 144.6 ± 3.6 vs. SOLF, 117.6 ± 3.2; Fig. 4B and D). In contrast, UOLF failed to inhibit LTD induction (SD, 148.9 ± 5.0 vs. UOLF, 138.6 ± 3.3; ns; 10 min after HFS, Fig. 4B and C), but fully abolished LTP maintenance (last 10 min of the recording; SD, 144.6 ± 3.6 vs. UOLF, 107.9 ± 2.8; Fig. 4B and D). In summary, our results show that LTP induction is specifically impaired by SOLF while UOLF interferes with LTD maintenance.

In regard to NMDA-induced LTD, two-way repeated measures ANOVA (from the beginning until before HFS application) indicated statistical significance in diet, time and interaction diet x time ($P < 0.01$, $P < 0.01$ and $P < 0.001$, respectively; post hoc analysis are shown in the graph). Fig. 5 shows that this mechanism was impaired by UOLF since long-lasting depression of the fEPSP triggered by NMDA was not observed in UOLF-treated mice (SD: 53.2 ± 3.5 vs. UOLF: 85.7 ± 3.3).

In contrast, SOLF mice displayed a stronger NMDA-LTD than controls (SD, 53.2 ± 3.5 vs. SOLF, 37.3 ± 2.9).

To further identify the extent to which NMDA responses were due either to synaptic plasticity or to an eventual toxic effect, HFS was applied 50 min after NMDA, once LTD was established (Fig. 5A). In control slices, HFS induced a re-potentiation of fEPSP until baseline values (109.0 ± 5.4, Fig. 5C), which was also observed in UOLF slices (99.8 ± 3.7, Fig. 5C), thus suggesting that HFS was able to produce synaptic plasticity. In contrast, HFS applied to SOLF slices did not evoke any change (65.9 ± 1.5) compared to the Control and UOLF groups (Fig. 5C). This result would indicate that SOLF-treated HIP present less plasticity, which could be due, at least in part, to the apparent toxic effect of the combination of lauric and palmitic acids contained in SOLF (see LDH cytotoxic assay; Fig. 2, Supplementary Material).

### 3.5. Plasma biochemistry

Biochemical data from Study 1 have been previously reported (Plaza et al., 2019) and Table 1 shows data corresponding to Study 2. An increase in NEFA values was observed only in SOLF-fed mice ($P < 0.01$), whereas the increase in leptin and insulin was statistically significant only in UOLF mice ($P < 0.05$ and $P < 0.001$, respectively). No changes in GLP, resistin, IL6, TNFα and MCP1 (data not shown), triglycerides and adiponectin were found. BW gain and food intake are shown in Supplementary Material (Fig. 1).
Fig. 3. Effect of 8-week SOLF and UOLF on mRNA levels of glutamate receptors, PPAR, and hormone receptors in adolescent and adult mice hippocampus. Panels A–E illustrate the effect of UOLF and SOLF on gene expression of GLU receptor subunits (Grin2a: Diet, $F_{(2,34)} = 2.988, P < 0.05$; age, $F_{(1,34)} = 3.113, P < 0.01$; interaction, $F_{(2,34)} = 2.163, P = 0.063$; Grin2b: Diet, $F_{(2,30)} = 2.998, P < 0.05$; age, $F_{(1,30)} = 1.591, P = 0.219$; interaction, $F_{(2,30)} = 1.521, P = 0.221$). mRNA levels corresponding to PPARα, PPARγ, leptin, insulin and adiponectin receptors appear illustrated in panels I, J, K, L, M and N, respectively (Pparg: Diet, $F_{(2,28)} = 9.552, P < 0.001$; age, $F_{(1,28)} = 10.522, P < 0.001$; interaction, $F_{(2,28)} = 5.712, P < 0.001$, followed by Bonferroni post hoc test; * $P < 0.05$ adolescent SD vs. adolescent SOLF and adult SD vs. adult UOLF; # $P < 0.05$ adolescent UOLF vs. adult UOLF). Values are means ± SEM (adolescent mice, $n = 6$ for SD, $n = 7$ for UOLF and SOLF groups; adult mice, $n = 6$ for SD, $n = 7$ for UOLF and SOLF groups). Effect of SOLF/UOLF on GluA1, GluN2A and GluN2B receptor subunit protein levels are shown in panels 3 F, 3 G and 3 H, respectively.
The possibility that diet composition might be an obesity-independent triggering factor for cognitive impairment is stressed by authors have recently shown that acute exposure (7–9 days) to HFD during juvenility is sufficient to impair HIP functions depending on the intake of saturated fat, might contribute to the effects observed here. In any case, the influence of HIP insulin sensitivity on spatial memory is a controversial matter since HFD has a different impact on HIP insulin sensitivity in males and females, which is not accompanied by intersex differences in terms of spatial memory and HIP excitability (Underwood and Thompson, 2016). We want to emphasize, that, in the current study, we have decided to use only males to minimize the number of animals, but new research aimed at characterizing sex differences concerning SOLF/UOLF effects in cognition and synaptic plasticity will be necessary to properly characterize this issue.

Another circumstance that could account for cognitive deficits is the development of HIP leptin resistance triggered by SOLF. This is a relevant issue considering that leptin has been shown to promote HIP–development of HIP leptin resistance triggered by SOLF. This is a relevant issue considering that leptin has been shown to promote HIP–

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Fig. 4. Synaptic efficacy and long-term potentiation in hippocampus slices after 8-week SOLF and UOLF treatment. (A) Graph plots show stimulus-response curves (means ± S.E.M. of fEPSP slopes vs. stimulus intensity) to measure baseline synaptic responsiveness in HIP slices from SD (white circles, n = 8), SOLF (black circles, n = 10) and UOLF (grey circles, n = 12) treated animals. (Repeated measures ANOVA-2 diet, F(2,30) = 3.107, P < 0.05; intensity, F(11,110,45.45) = 74.81, P < 0.001; interaction, F(22,360) = 2.994, P < 0.01, followed by Bonferroni post hoc test, * P < 0.05 and **P < 0.01 SD vs. SOLF). (B) After 20 min of baseline recording, four trains of HFS (indicated by four arrows) were applied to slices from both SD (white circles, n = 9), SOLF (black circles, n = 8) and UOLF-treated animals (grey circles, n = 10). Comparison was made by repeated measures ANOVA-2 (Diet, F(2,23) = 6.468, P < 0.05; time, F(190,2507) = 13.93, P < 0.0001; interaction, F(218,2507) = 2.123, P < 0.001 followed by Bonferroni post hoc test * P < 0.001, * * P < 0.001 and * * * P < 0.001 SD vs. SOLF and **P < 0.01 and ## P < 0.001 SD vs. UOLF). C and D show the bars representing the mean ± S.E.M. of fEPSP measured for 5 min after 10 min and 90 min of HFS. (ANOVA-1 F(2172) = 33.05, P < 0.001 and F(2132) = 67.79, P < 0.001, respectively, followed by Bonferroni post hoc test; * * * P < 0.001 SD vs. SOLF and SD vs. UOLF; ** * * P < 0.001 SOLF vs. UOLF). Upper traces show representative recordings from one of each type of experiment (a and b show the time points corresponding to each curve). No more than 1 slice/mouse was used in each experiment.

damage in absence of obesity/overweight (Beilharz et al., 2015). It has to be highlighted that, under our experimental conditions, SOLF mice displayed elevated HOMA indexes (Plaza et al., 2019), a circumstance that might have an impact on memory (Rom et al., 2019). In fact, memory/learning deficits have been shown to be linked to brain insulin resistance (Grillo et al., 2015), a condition that can prematurely develops during HFD interventions before obesity is established (Vinuesa et al., 2016). It has to be noted that, in our study, plasma insulin levels were higher in UOLF than in SOLF mice, a data that needs to be interpreted cautiously considering that mice were not fasted before blood sampling. Therefore, a certain degree of insulin resistance, associated to the intake of saturated fat, might contribute to the effects observed here. Another circumstance that could account for cognitive deficits is the development of HIP leptin resistance triggered by SOLF. This is a relevant issue considering that leptin has been shown to promote HIP-dependent learning and memory (Van Doorn et al., 2017) and to regulate HIP synaptic transmission (Moult et al., 2010; Moult and Harvey, 2008).
In fact, leptin-insensitive mice have been shown to display a significant impairment of both LTP and LTD (Li et al., 2002; Winocur et al., 2005), and a desensitization of the protein kinase B (Akt) pathway coupled to the protein kinase C (PKC) pathway (Li et al., 2002; Winocur et al., 2005). Moreover, HIP leptin receptors (LepR) have been detected in adolescent mice consuming HFD and displaying deficits in spatial memory and moderate hyperleptinemia (Valladolid-Acebes et al., 2013). In the same vein, engineered mice lacking LepR specifically in astrocytes display impaired BST and LTD in the HIP (Naranjo et al., 2019). The possibility that SOLF would promote leptin resistance seems unlikely as plasma leptin levels in adolescent SOLF mice were lower than in their UOLF counterparts, in accordance with previous findings showing that SOLF represses leptin gene expression in visceral WAT (Plaza et al., 2019). However, this possibility cannot be fully discarded inasmuch as diets enriched in saturated fat have been shown to disrupt the blood-brain barrier (Hsu and Kanoski, 2014). Thus, it can be speculated that leptin access to the brain would be increased by SOLF with the consequent development of leptin resistance.

The electrophysiological data suggest that the impairment of spatial memory in SOLF-treated animals is related, at least in part, to the effect of saturated FAs on HIP plasticity and particularly on BST, as previously observed in a study carried out with a classical HFD (Valladolid-Acebes et al., 2012). A surprising result was the shortening of LTP triggered by both SOLF and UOLF, even though SOLF was more potent than UOLF in inhibiting LTP induction. This finding is coherent with studies carried out in animals treated with HFD (Hao et al., 2016), as well as with in...
vitro assays showing that HIP slices treated with palmitic acid display a partial inhibition of LTP (Contreras et al., 2017), and confronts with the lack of effect of HFD identified in other studies (Valladolid-Acebes et al., 2012). In any case, the negative impact of UOLF on LTP duration and BST strongly suggests that unsaturated FAs may also have a negative impact on HIP plasticity. Nevertheless, the molecular mechanisms that account for SOLF and UOLF effects in LTP seem to be different, since SOLF affected the induction of the phenomenon whereas UOLF impaired its maintenance. Wong et al. (1989) observed differences between unsaturated and saturated fat-enriched diets in synaptic plasticity mechanisms and observed that a HFD containing both lauric and myristic acids reduced the phosphorylation of the substrate protein F1 (aka GAP43), a factor that accounts for HIP synaptic plasticity (Linden and Routtenberg, 1989). On this basis, one could speculate that a deficient activity of PKC signaling pathways might account for LTP impairment triggered by SOLF.

Regarding LTD, our results are striking considering that it was inhibited by UOLF but apparently potentiated by SOLF. The effect of UOLF, which is similar to that provoked by classical HFDs (Hwang et al., 2010; Valladolid-Acebes et al., 2012), would suggest that unsaturated FAs impair this plasticity mechanism. As changes in NR2A/NR2B ratios have been related to LTD thresholds (Xu et al., 2009), the modulation of NMDAR subunits detected in SOLF-treated mice could underline changes in synaptic transmission and plasticity. In any case, the effect of SOLF in LTD is difficult to interpret since HFS was unable to re-potentiate the fEPSP slope, as it did in control and UOLF-treated animals. This condition suggests a toxic effect of SOLF, able to exacerbate NMDA-evoked LTD. Such a possibility would be compatible with cytotoxicity detected in HIP slices incubated with a combination of lauric and palmitic acids, used in a proportion similar to that contained in SOLF (Fig. 2, Supplementary Material).

The electrophysiological and behavioural findings could be related to the down-regulation of Grin2A and Grin2B gene expression triggered by SOLF and UOLF. Such an adaptive regulation is coherent with the specific role of GluN2A and GluN2B in both LTD and LTD (Foster et al., 2010; Kellermayer et al., 2018; Liu et al., 2004; Shipton and Paulsen, 2014). In fact, GluN2A deletion mitigates HIP LTP and impairs spatial learning (Kannagara et al., 2015; Kiyama et al., 1998; Sakimura et al., 1995). In addition, changes in NMDAR density have been detected after synaptic plasticity induction as well as by spatial memory formation (Baez et al., 2018). Some authors point to the increase in the synaptic GluN2A/GluN2B ratio as a stabilizer of synaptic changes, contributing to memory consolidation, particularly concerning spatial representation (Baez et al., 2018). Our data are coherent with this idea and would suggest that modulation of NMDAR subunits by SOLF might underlie the deleterious effect of unsaturated FAs on spatial memory. It must be noted that the inhibition of Grin2A and Grin2B expression was not accompanied by a significant decrease of the corresponding GluN2A and GluN2B proteins. This circumstance could be due to the fact that protein levels change more slowly than the corresponding mRNA (Vogel and Marcotte, 2013) or even to a poor efficiency of translation mechanism, previously reported by other authors (Bouet et al., 2017; Schindler et al., 1990). In addition, the stability observed in AMPAR subunit mRNA levels (Gria1 and Gria2) also points to the lack of effect of the diets on AMPAR subunit content. The lack of effect of SOLF/UOLF on Gria1 levels contrasts with the results reported by Spinelli et al. (2017). Although we cannot provide any precise interpretation to explain this difference, we could speculate that SOLF/UOLF and the diet used by Spinelli et al., probably affect GLU turnover (Valladolid-Acebes et al., 2012) differently and trigger a distinct regulation of GLU receptor subunits, as a consequence of specific adaptive compensatory mechanisms. Unfortunately, we lack data regarding the influence of SOLF/UOLF on HIP GLU uptake kinetics that would have allowed proper discussion of this issue.

Finally, the inhibition of PPARY gene expression suggests that the impairment of synaptic plasticity induced by SOLF could be related to PPARY modulation, in addition to the decrease in NMDA subunits 2A and 2B. In this regard, our previous studies have shown that commercial HFD containing lard decreases NMDA2B gene expression concomitantly with deficits in spatial learning (Valladolid-Acebes et al., 2011). Moreover, the activation of PPARY has been demonstrated to ameliorate spatial memory deficits (Chen et al., 2016). For these reasons, further studies using pharmacological tools to investigate whether NMDA and PPARY agonists prevent/reverse SOLF-evoked spatial memory deficits would be necessary.

Taken together, our data suggest that changes of synaptic plasticity could be mainly related to post-translational modifications of GLU receptors that modulate their functionality; otherwise, one can speculate that changes in plasma levels of insulin and leptin, eventually leading to insulin and/or leptin resistance, might also be pivotal in modulating both HIP synaptic plasticity and short-term memory, particularly in SOLF-treated mice (Harvey, 2007; Harvey et al., 2006). Moreover, PPARY might also play a role in SOLF effects. In conclusion, our study shows that diets enriched with either saturated or unsaturated FAs modulate spatial HIP-dependent spatial memory through modulation of synaptic transmission and plasticity mechanisms in this area.

Funding

Ministerio de Ciencia e Innovación (BFU2016–78556-R, BFU2017–82565-C21-R2), European Regional Development Fund, Universidad Nacional de Educación a Distancia (UNED) and Fundación Universitaria San Pablo-CEU. A.P. is supported by a grant from Ministerio de Ciencia e Innovación (BFU2016–78556-R). J.F.-P. and A.C. are supported by grants from Fundación Universitaria San Pablo-CEU. A.B. S.-M. is supported by the Youth Employment Initiative (YEI) de Comunidad Autónoma de Madrid.

Acknowledgements

We thank J.M. Garrido, I. Bordallo and J. Bravo for their assistance with animal care.

Disclosure statement

The authors have nothing to disclose.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2021.105429.

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