



Adipocyte cannabinoid CB1 receptor deficiency alleviates high fat diet-induced memory deficit, depressive-like behavior, neuroinflammation and impairment in adult neurogenesis



Juan Suárez^{a,*}, Patricia Rivera^b, Alejandro Aparisi Rey^c, Margarita Pérez-Martín^d, Sergio Arrabal^a, Fernando Rodríguez de Fonseca^a, Inigo Ruiz de Azua^{c,e,1}, Beat Lutz^{c,e,*,1}

^a Unidad de Gestión Clínica de Salud Mental, Instituto de Investigación Biomédica de Málaga (IBIMA), Hospital Regional Universitario de Málaga, Málaga, Spain

^b Department of Endocrinology, Fundación Investigación Biomédica del Hospital Infantil Universitario Niño Jesús, Madrid, Spain

^c Institute of Physiological Chemistry, University Medical Center of the Johannes Gutenberg University of Mainz, Mainz, Germany

^d Departamento de Biología Celular, Genética y Fisiología, IBIMA, Universidad de Málaga, Málaga, Spain

^e German Resilience Center (DRZ), Mainz, Germany

ARTICLE INFO

Keywords:

Adipocyte CB1
Obesity
Inflammation
Hippocampus
Memory
Neurogenesis

ABSTRACT

Background: Obesity is a low-grade inflammation condition that facilitates the development of numerous comorbidities and the dysregulation of brain homeostasis. Additionally, obesity also causes distinct behavioral alterations both in humans and rodents. Here, we investigated the effect of inducible genetic deletion of the cannabinoid type 1 receptor (CB1) in adipocytes (Ati-CB1-KO mice) on obesity-induced memory deficits, depressive-like behavior, neuroinflammation and adult neurogenesis.

Methods: Behavioral, mRNA expression and immunohistochemical studies were performed in Ati-CB1-KO mice and corresponding wild-type controls under standard and high-fat diet.

Results: Adipocyte-specific CB1 deletion reversed metabolic disturbances associated with an obese condition confirming previous studies. As compared to obese mice, the metabolic amelioration in Ati-CB1-KO mice was associated with an improvement of mood-related behavior and recognition memory, concomitantly with an increase in cell proliferation in metabolic relevant neurogenic niches in hippocampus and hypothalamus. In mutant mice, these changes were related to an increased neuronal maturation/survival in the hippocampus. Furthermore, CB1 deletion in adipocytes was sufficient to reduce obesity-induced inflammation, gliosis and apoptosis in a brain region-specific manner.

Conclusions: Overall our data provide compelling evidence of the physiological relevance of the adipocyte-brain crosstalk where adipocyte-specific CB1 influences obesity-related cognitive deficits and depression-like behavior, concomitantly with brain remodeling, such as adult neurogenesis and neuroinflammation in the hippocampus and hypothalamus.

Abbreviations: ARC, arcuate nucleus of hypothalamus; Ati-CB1-KO mice, mice with conditional adipocyte-specific CB1 gene deletion induced by tamoxifen administration; BrdU, 5-bromo-2'-deoxyuridine; CA1/3, field 1/3 of Cornu Ammonis; CB1, cannabinoid type 1 receptor; DIO, diet-induced obesity; DG, dentate gyrus; gcl, granular cell layer; FS, forced swim test; GFAP, glial fibrillary acidic protein; HFD, high-fat diet; Iba-1, ionized calcium-binding adapter molecule 1; IL-6, interleukin-6; ml, molecular layer; NeuN (Rbfox-3), RNA binding protein fox-1 homolog 3; NOR, novel object recognition p1 polymorphic cell layer; PVH, paraventricular nucleus of hypothalamus; SGZ, subgranular zone of dentate gyrus; SL-M, stratum lacunosum-moleculare; SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum; SD, standard diet; VMH, ventromedial nucleus of hypothalamus

* Corresponding author at: UGC Salud Mental, IBIMA, Hospital Regional Universitario de Málaga, Universidad de Málaga. Avenida Carlos Haya 82, Pabellón de Gobierno, 29010, Málaga, Spain.

** Corresponding author at: Institute of Physiological Chemistry, University Medical Center of the Johannes Gutenberg-University of Mainz, Duesbergweg 6, 55099 Mainz, Germany.

E-mail addresses: juan.suarez@ibima.eu (J. Suárez), beat.lutz@uni-mainz.de (B. Lutz).

¹ These authors share senior authorship.

<https://doi.org/10.1016/j.psyneuen.2019.104418>

Received 16 April 2019; Received in revised form 14 July 2019; Accepted 22 August 2019

0306-4530/© 2019 Elsevier Ltd. All rights reserved.

1. Introduction

Obesity is defined as a chronic low-grade inflammatory disease contributing to metabolic syndrome. Its etiology is linked among others to an immune response and subsequent secretion of pro-inflammatory cytokines that affects peripheral and central tissues, including hypothalamus and hippocampus (Purkayastha and Cai, 2013a). Recent investigations have identified that brain inflammation results in impairment of neural stem cell proliferation and differentiation (Purkayastha and Cai, 2013b).

Obesity is also associated with distinct alterations in brain function, such as cognitive impairment (Dye et al., 2017) and psychiatric disorders including depression and anxiety (Garipey et al., 2010; Hryhorczuk et al., 2013; Capuron et al., 2017). Several processes have been considered to be responsible for these obesity-related mood disorders such as metabolic signals (Hryhorczuk et al., 2013), inflammation (Lasselin and Capuron, 2014) and most recently senescence (Ogrodnik et al., 2019). Nevertheless, the underlying mechanisms still remain elusive.

In obesity, adipocytes undergo both hyperplasia and hypertrophy, accompanied by macrophage infiltration and M1 polarization state (Lumeng et al., 2007). Adipose tissue macrophages are the main source of cytokines in response to obesity and, therefore, mainly responsible for inflammation and insulin resistance. Increasing evidence indicate that hypothalamic inflammation contributes to the development and progression of obesity and insulin resistance (Jais and Brüning, 2017). Interestingly, hypothalamic inflammation occurs prior to body weight gain, simultaneously with microglial activation (Thaler et al., 2012). In this sense, ablation of microglia abrogates high-fat diet (HFD) induced hypothalamic inflammation and neuronal stress, and limits diet-induced reduced energy expenditure, hyperphagia and body weight gain (André et al., 2017; Valdearcos et al., 2014, 2017). Activated hypothalamic microglia produce pro-inflammatory cytokines, such as TNF- α , IL-6 and IL-1 β (Nakanishi et al., 2007; Lamberts et al., 2009). In addition, along with the reactive microgliosis, HFD causes astrocyte activation in the hypothalamus, which persists into later obesity (Thaler et al., 2012; Buckman et al., 2014). Finally, long-lasting HFD leads to apoptosis of hypothalamic neurons due to chronic inflammatory responses (Moraes et al., 2009). Furthermore, obesity-induced inflammation has been associated with reduction of adult neurogenesis in hypothalamic and hippocampal neurogenic niches (Lindqvist et al., 2006; Boitard et al., 2014; Lee et al., 2012; McNay et al., 2012). Congruently, IL-6 administration or overexpression causes a decrease in hippocampal neurogenesis and memory formation (Donzis and Tronson, 2014).

Obesity is associated with an overactive endocannabinoid (eCB) system which plays a critical role in energy balance (Osei-Hyiaman et al., 2006; Quarta et al., 2010; Silvestri and Di Marzo, 2013), as well as other related physiological responses, such as inflammation and endocrine regulation (Pagotto et al., 2006; Stella, 2010; Cluny et al., 2012). Emerging evidence suggests that eCB-mediated control of energy homeostasis results from a crosstalk between central and peripheral processes (Malcher-Lopes et al., 2006; Ruiz de Azua and Lutz, 2019). Recently, we reported that adipocyte CB1 plays a crucial role in regulating energy homeostasis through signaling mechanisms involving adipocytes, immune cells and sympathetic nervous system (Ruiz de Azua et al., 2017). Adipocyte-specific CB1 deletion was sufficient to reduce body weight and fat mass, improve insulin sensitivity, and enhance brown adipose tissue thermogenesis. These changes were associated with a profound remodeling of adipose tissue, including an increase in alternatively activated macrophages concomitantly with an enhanced sympathetic tone (Ruiz de Azua et al., 2017).

The eCB system also regulates inflammation and proliferation in the brain, which may be partly mediated by the presence of CB1 in astrocytes (García-Ovejero et al., 2013; Metna-Laurent and Marsicano, 2015). Central immune system-related function is modulated by CB1

activation in resident microglial cells and astrocytes (Stella, 2010). Thus, *in vitro* CB1 stimulation inhibits the release of proinflammatory cytokines from reactive astrocytes (Ortega-Gutiérrez et al., 2005). Besides, CB1 signaling regulates proliferation in specific neurogenic niches, as well as cell survival (Rivera et al., 2011; Maccarrone et al., 2014; Zimmermann et al., 2018). Remarkably, pharmacological CB1 blockage in diet-induced obesity (DIO) models resulted in metabolic improvement and cell proliferation increase in the subgranular zone (SGZ) of the dentate gyrus, but a decrease in the subventricular zone (SVZ) of the lateral ventricles and the hypothalamus (Rivera et al., 2011). Similarly, blocking CB1 responses in HFD models abolished the diet preference and astrocyte proliferation in the hypothalamus (Higuchi et al., 2010), indicating that CB1 inhibition protects against obesity by reducing astrogliosis.

Here, we investigated the effect of adipocyte-specific CB1 deletion (Ati-CB1-KO) on obesity-induced cognitive decline and depression-like behaviors. Furthermore, we examined whether these behaviors were associated with improvements in adult neurogenesis and neuroinflammation in the hippocampus and hypothalamus.

2. Material and methods

2.1. Ethics statement

Experimental procedures with animals were carried out following the recommendations of the European Communities directive 2010/63/EU regulating the care and use of laboratory animals. The protocols were approved by the Ethic and Research Committee of Rhineland-Palatinate (Germany) (AZ.: 23177-07/G10-1-38).

2.2. Animals

Ati-CB1-KO mice (specific deletion of CB1 gene in adipocytes) and their littermate controls ($CB1^{floxed/floxed}$, named Ati-CB1-WT) were generated as described previously (Ruiz de Azua et al., 2017). Male mice at 6–7 weeks were housed individually and maintained in standard conditions. Ati-nuGFP and CaMK-nuGFP mice were generated by crossing AdipoqCreER^{T2} and CaMKIIICre with ROSA26-CAG-LSL-Sun1-sfGFP-myc mice (Quarta et al., 2010; Ruiz de Azua et al., 2017). See also Supplementary information.

2.3. Food/caloric intake and body weight

Mice ($n = 12$ – 16 /group) were fed ad libitum for 26 weeks with a standard diet (SD) containing 12.3 kJ/g (11/36/53% of fat/protein/carbohydrate; cat. no. V1126-000, Ssniff) and a high-fat diet (HFD) containing 21.1 kJ/g (60/17/23% of fat/protein/carbohydrate; cat. no. C1090/60, Altromin). The accumulated food/caloric intake and the body weight gain were weekly measured for 26 weeks (Fig. 1A).

2.4. Specific induction of conditional CB1 deletion in adipocytes

After 17 weeks on both diets, tamoxifen was intraperitoneally (i.p.) administered (1 mg/day) for 10 days (Fig. 1A) in all mice. Tamoxifen preparation was described previously (Ruiz de Azua et al., 2017). Thus, four experimental groups were generated ($n = 6$ – 8): SD Ati-CB1-WT, SD Ati-CB1-KO, HFD Ati-CB1-WT and HFD Ati-CB1-KO.

2.5. Behavior

2.5.1. Novel object recognition (NOR) test

The capacity to memorize a previously encountered object was tested in 2 consecutive steps. During the training phase, mice explored two identical objects for 5 min. One hour later, one of the familiar objects was substituted by a new object, and mice explored them for another 5 min. The exploration time of both objects was scored and a

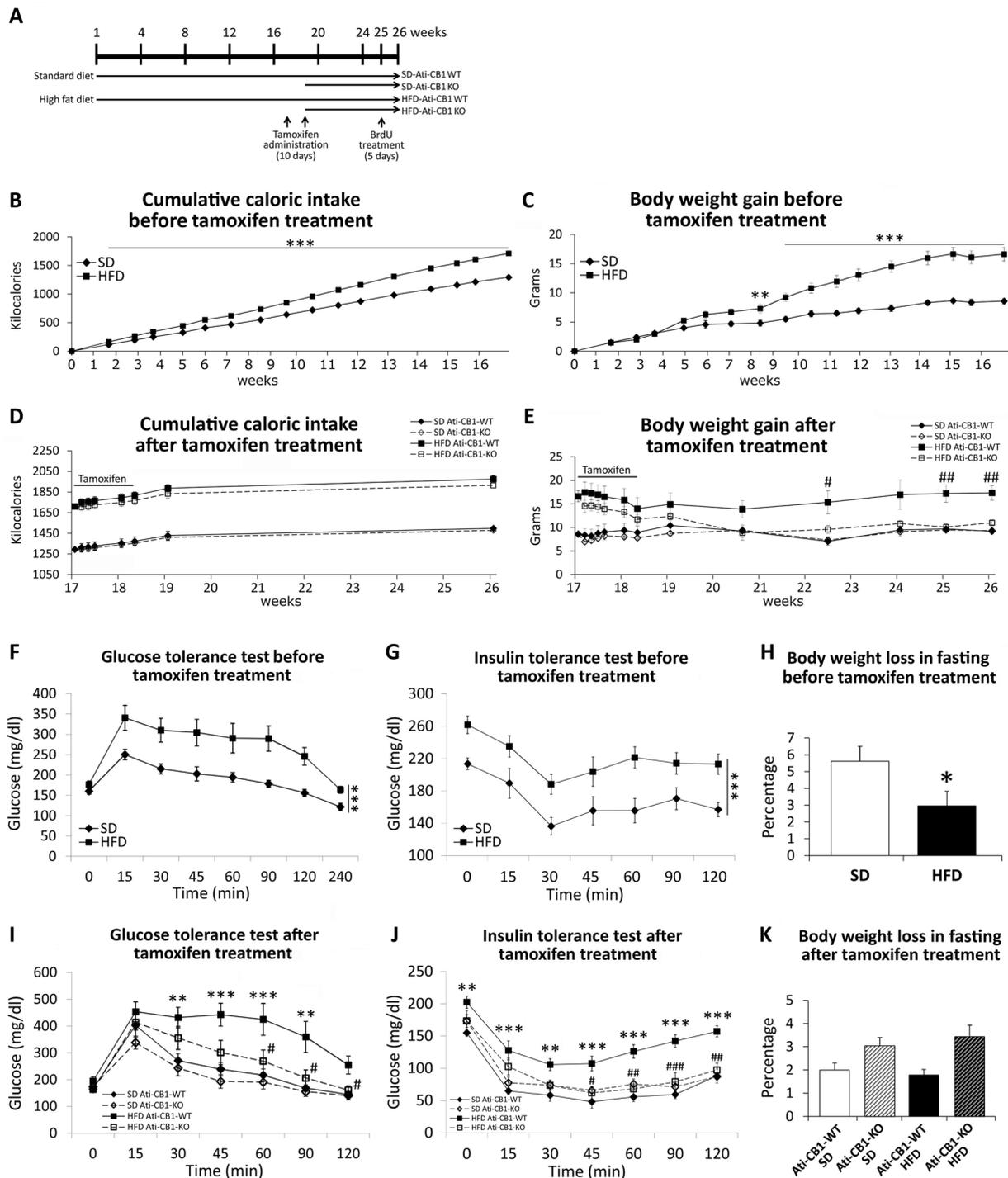


Fig. 1. A) Experimental design used for high-fat diet-induced obesity (DIO), induction of conditional adipocyte-specific CB1 gene deletion by tamoxifen administration for 10 days and 5'-bromo-2'-deoxyuridine (BrdU) injections for 5 days. B–E) Effect of SD and HFD on cumulative caloric intake and body weight gain before tamoxifen treatment, over 16 weeks of feeding (B, C), and after the induction of adipocyte-specific CB1 gene deletion by tamoxifen administration (D, E). F–K) Effect of SD and HFD on glucose tolerance (F, I), insulin sensitivity (G, J) and percentage of body weight loss (H, K) in 6-h fasted mice 2 weeks before (DIO mice) and 4 weeks after the induction of adipocyte-specific CB1 gene deletion (Ati-CB1-KO mice). The histograms and points indicate the mean ± SEM (12–16 animals per diet group, 6–8 animals per genotype subgroup). Bonferroni's test: $^{*}/^{**}/^{***}P < 0.05/0.01/0.001$ vs. SD-fed mice and SD-fed Ati-CB1-WT mice; $^{#}/^{##}/^{###}P < 0.05/0.01/0.001$ vs. HFD-fed Ati-CB1-WT mice. Student *t*-test in H.

discrimination index was calculated as follows: (time exploring familiar object/time exploring both objects) x 100.

2.5.2. Forced swim (FS) test

The natural aversion of mice to water was used to evaluate depressive-like behavior. Mice were placed during 6 min in a glass beaker

filled with water. The time of swimming and floating was recorded and used as a proxy of depressive symptoms. Thus, “despair” behavior is usually characterized by a reduction in swimming accompanied by increased floating.

2.6. Glucose and insulin tolerance tests

Two weeks before and 4 weeks after the induction of adipocyte-specific CB1 deletion, we have performed a glucose and insulin tolerance tests. In brief, mice were starved for 6 h previous starting the glucose tolerance tests. Glucose levels were determined at 0 (basal levels), 15, 30, 45, 60, 90 and 120 min after a glucose overload (2 g/kg, i.p.). Regarding insulin tolerance tests, we used different experimental conditions before and after tamoxifen treatment. Two weeks before tamoxifen, the insulin tolerance test was performed using an insulin administration of 0.75 IU/kg, mice were starved for 4 h, and glucose levels were measured. Four weeks after the induction of adipocyte-specific CB1 deletion, we repeated the insulin tolerance test using a low dose of insulin (0.5 IU/kg, *via.i.p.*) and mice were starved for 6 h.

2.7. BrdU administration

5'-bromo-2'-deoxyuridine (BrdU, cat. no. B5002, Sigma-Aldrich) was administrated at 70 mg/kg (i.p.), twice per day at 10-hs intervals, for five consecutive days during the week 26 (Rivera et al., 2011). Brains were collected 12 h after the last injection (Fig. 1A).

2.8. Sample collection

Mice were sacrificed 8 weeks after tamoxifen administration. Brain, epididymal white adipose tissue (eWAT) and skeletal muscle were dissected out and stored at -80 °C for mRNA expression analyses. Hippocampi (2.4 mm, starting at Bregma -1.6 mm) and hypothalami (1.5 mm, starting at Bregma -0.60 mm) were precisely punched. A second batch of mice were transcardially perfused (buffered-4% paraformaldehyde), and brains were cut into 30- μ m-thick coronal sections for immunohistochemistry.

2.9. RNA isolation and real-time PCR analysis

We performed real-time PCR (Applied Biosystems), as was described previously (Ruiz de Azua et al., 2017). RNA was extracted following Trizol method (Sigma; cat. no. 12183-555). After reverse transcriptase reaction (1 μ g RNA), quantitative RT-PCR was performed in an Applied Biosystems® 7300 Real-Time PCR Detection System and the FAM-dye label format for the TaqMan® Gene Expression Assays (Applied Biosystems) (Table S1 and Supplementary information). Values were normalized to β -glucuronidase (Gusb) mRNA expression.

2.10. Immunohistochemistry and double immunofluorescence

Samples (either deparaffinized adipose tissue sections or brain floating sections) were incubated overnight at 4 °C with rat anti-BrdU (1:2000; Accurate Chemical & Scientific; OBT0030 F) or a rabbit anti-GFP (1:500, STRATAGENE; 240142) antibody (Rivera et al., 2011); or at room temperature with mouse anti-NeuN (1:500, Millipore, MAB377), mouse anti-GFAP (1:500, Sigma; G3893), rabbit anti-Iba-1 (1:1000, Wako, 019-19741), and mouse anti- β -tubulin (1:5000; Promega, G7121) [36]. For fluorescence, sections were incubated in goat anti-rat IgG labeled with Alexa Fluor® 488 (1:1000; Molecular Probes, Invitrogen, A11006) and donkey anti-mouse IgG labeled with Alexa Fluor® 594 (1:1000; Molecular Probes; A21203), and visualized with a confocal microscope (TCS NT; Leica Microsystems).

2.11. Quantification of BrdU, β 3-tubulin and Iba-1-immunoreactive cells

Cells were manually counted from -1.6 to -4.1 mm Bregma levels in the hippocampus [subgranular zone (SGZ) of the dentate gyrus (DG), CA3 and CA1 areas], and from -0.6 to -2.1 mm Bregma levels in the hypothalamus [paraventricular (PVH), ventromedial (VMH) and arcuate (ARC) nuclei]. Quantification was expressed as the average

number of BrdU, β 3-tubulin and Iba-1-ir cells/area (mm²). The number of cells with double immunofluorescence staining for BrdU and β 3-tubulin was expressed as percentage with respect to total BrdU + cells.

2.12. Quantification of NeuN and GFAP immunoreactivity

Densitometric quantification of the immunoreactivity of the same hippocampal and hypothalamic areas was determined using the analysis software ImageJ 1.38X (NIH, USA). We considered the following hippocampal layers: strati oriens (SO), pyramidale (SP), radiatum (SR), lucidum (SL) and lacunosum-moleculare (SL-M), and molecular (ml) granular (gcl), and polymorphic (pcl) cell layers.

2.13. Statistical analysis

All data are shown as mean \pm SEM. Levene normality tests indicated that all data followed a Gaussian distribution ($P > 0.1$), so we selected parametric statistical tests. Differences were evaluated using repeated measures, two and three-way ANOVA being time, diet (SD and HFD) and genotype (WT and KO) as factors, following multiple comparisons by corrected Bonferroni's or Sidak's test when appropriate. Simple effect analysis was systematically performed in case of interaction or main effect. Student's unpaired *t*-test was only performed when two single groups were compared. $P < 0.05$ was considered significant.

3. Results

3.1. Effect of adipocyte-specific CB1 deletion on caloric intake and body weight

First, we have analyzed the metabolic effect of adipocyte-specific CB1 deficiency in lean and HFD-induced obese mice. Before tamoxifen-induced recombination, significant interactions between diet (SD vs HFD) and time were found in cumulative caloric intake ($F_{16,406} = 42.16$, $P < 0.0001$) and body weight gain ($F_{16,388} = 11.96$, $P < 0.0001$). A diet effect on cumulative caloric intake ($F_{1,406} = 1968.5$, $P < 0.0001$) and body weight gain ($F_{1,388} = 293.08$, $P < 0.0001$) was also observed. Accordingly, HFD-fed mice consumed more caloric intake ($***P < 0.001$; from second week on) and showed a higher body weight gain (week 8, $**P < 0.01$; from week 8 on, $***P < 0.001$) compared to SD-fed mice (Fig. 1B, C).

After tamoxifen-induced adipocyte-specific CB1 ablation, significant interactions between diet and genotype were found in body weight gain ($F_{1,20} > 5.03$, $P < 0.05$), but not anymore in caloric intake. A diet effect was still observed after tamoxifen treatment on cumulative caloric intake ($F_{1,49} = 493.72$, $P < 0.0001$) and body weight gain ($F_{1,85} = 79.2$, $P < 0.0001$), being higher in HFD-fed Ati-CB1-WT mice than in SD-fed mice. We also detected a genotype effect on cumulative caloric intake ($F_{1,77} = 4.8$, $P = 0.031$) and body weight gain ($F_{1,132} = 39.71$, $P < 0.0001$) in HFD-fed mice, but not in SD-fed mice. When Bonferroni analysis was applied, HFD-fed Ati-CB1-KO mice showed a reduced body weight gain as compared to HFD-fed Ati-CB1-WT mice (week 22, $^{\#}P < 0.05$; week 25 and 26, $^{\#\#}P < 0.01$) (Fig. 1D, E), but no significant differences were found in caloric intake.

3.2. Effect of adipocyte-specific CB1 deletion on glucose and insulin tolerance

Additionally, we have also analyzed the glucose homeostasis in these mice. Two weeks before tamoxifen treatment, we found a diet effect on glucose tolerance ($F_{1,112} = 60.64$, $P < 0.0001$) and insulin sensitivity ($F_{1,168} = 49.52$, $P < 0.0001$). HFD-fed mice showed increases in the circulating glucose levels during glucose (Fig. 1F) and insulin tolerance tests (Fig. 1G). This impairment in glucose homeostasis was associated with a lower body weight loss in HFD-fed mice

during fasting (**P* < 0.05) (Fig. 1H).

Four weeks after adipocyte-specific CB1 ablation, we repeated the glucose and insulin tolerance tests. Significant interactions were found between diet and genotype in blood glucose levels ($F_{1,126} > 10.41$, $P < 0.01$). Diet effects on glucose tolerance ($F_{1,98} = 52.84$, $P < 0.0001$) and insulin tolerance ($F_{1,98} = 33.49$, $P < 0.0001$) showed higher levels of glucose in HFD-fed Ati-CB1-WT mice than in SD-fed Ati-CB1-WT mice, but not between SD-fed and HFD-fed Ati-CB1-KO mice. Genotype effects on glucose ($F_{1,98} = 22.93$, $P < 0.0001$) and insulin tolerance ($F_{1,98} = 22.54$, $P < 0.0001$) indicated lower levels of glucose in HFD-fed Ati-CB1-KO mice, but not in SD-fed Ati-CB1-KO mice. Bonferroni analysis showed that the glucose intolerance and insulin resistance in the HFD-fed Ati-CB1-WT mice (**/**P* < 0.01/0.001 vs SD-fed Ati-CB1-WT mice) were normalized in the HFD-fed Ati-CB1-KO mice (#/#/#/#*P* < 0.05/0.01/0.001 vs HFD-fed Ati-CB1-WT mice) (Fig. 1I, J). A genotype effect on body weight loss during fasting was also found ($F_{1,20} = 10.5$, $P = 0.004$), with Ati-CB1-KO mice showing increased body weight loss (Fig. 1K).

3.3. Behavioral implications of adipocyte-specific CB1 deletion during DIO

During the training phase of the NOR test, all experimental groups explored similarly both objects [discrimination index (time exploring familiar object/total time exploration) = 50] (Fig. 2A). The statistical analysis of the discrimination index between training and retention phases revealed an interaction ($F_{3,22} = 3.715$, $P = 0.026$) and a time effect ($F_{1,22} = 67.14$, $P < 0.0001$). Moreover, a significant interaction between diet and genotype was observed in the discrimination index during the retention phase of NOR test ($F_{1,22} = 7.80$, $P < 0.001$). Interestingly, all experimental groups, except Ati-CB1-WT mice fed with HFD displayed a significant reduction of the discrimination index in the retention phase (SSS*P* < 0.001) (Fig. 2A). This reduction in the discrimination index reveals a proper memory formation, a process that is affected in Ati-CB1-WT mice fed with HFD. Depressive-like behavior

has been previously linked to obesity and is commonly analyzed by the FS test, whereby a decreased swimming and increased floating (helplessness) is usually associated with a depressive state. A significant interaction between diet and genotype was observed in swimming ($F_{1,22} = 4.74$, $P = 0.005$). In HFD-fed Ati-CB1-WT mice, swimming is significantly reduced (***P* < 0.01) (Fig. 2B). Interestingly, Ati-CB1-KO mice fed with the same diet reversed this phenotype (#*P* < 0.05) (Fig. 2B). Thus, adipocyte-specific CB1 deletion after DIO prevents depressive-like behavior and memory deficit, which can be associated with obesity.

3.4. CB1, Caspase-3 and IL-6 gene expression in metabolically relevant tissues

Diet effects on the CB1 mRNA expression were found in eWAT, skeletal muscle and hippocampus (Table 1). In particular, HFD-fed Ati-CB1-WT mice showed an increase in CB1 mRNA expression in eWAT and hippocampus, and a 3-fold decrease in skeletal muscle (Table 1). Genotype effects on CB1 mRNA expression were observed in eWAT and hypothalamus. As expected, 8 weeks after tamoxifen administration, specific induction of CB1 deletion in adipocytes caused a significant reduction of 70–85% in the CB1 mRNA expression in eWAT in a diet-independent manner, but not in other non-adipose tissues, such as skeletal muscle and hippocampus. Strikingly, HFD-fed Ati-CB1-KO mice also had a reduced CB1 mRNA expression in hypothalamus. We generated two reporter mouse lines (called Ati- and CaMKII-nuGFP mice, expressing nuclear membrane-tagged GFP-tagged after Cre-mediated recombination) to explore whether this reduction is an indirect effect of weight loss associated with adipocyte-specific CB1 deletion (Supplementary Information). In the Ati-nuGFP mice, we only found GFP-positive nuclei in adipocytes (eWAT, BAT), but none in the entire brain, including the hypothalamus (Fig. S1), in contrast to CaMKII-nuGFP mice. Therefore, the decreased CB1 expression in the hypothalamus is caused by an indirect effect of CB1 deletion in adipocytes.

Inflammation associated with long-term HFD leads to apoptosis of hypothalamic neurons (Moraes et al., 2009). Therefore, we assessed the local inflammatory processes and cell apoptosis in the hypothalamus and hippocampus by mRNA expression analysis of interleukin-6 (IL-6) and Caspase-3, respectively (Table 1). We found significant interactions between diet and genotype in the mRNA expression of Caspase-3 and IL-6 in the hippocampus. We also found a genotype effect on the hippocampal Caspase-3 mRNA expression. In this regard, the increased mRNA expression of Caspase-3 and IL-6 in the hippocampus of HFD-fed Ati-CB1-WT mice (**P* < 0.05 vs SD-fed Ati-CB1-WT mice) was completely reversed after tamoxifen-induced adipocyte-CB1 deletion (#/#/#*P* < 0.05/0.01 vs HFD-fed Ati-CB1-WT mice) (Table 1). In the hypothalamus, a diet effect was observed on the IL-6 mRNA expression, with HFD-fed Ati-CB1-KO mice showing a lower IL-6 mRNA expression (#*P* < 0.05 vs HFD-fed Ati-CB1-WT mice).

3.5. Cell proliferation and neuronal maturation in the subgranular zone of dentate gyrus and hypothalamus: BrdU and β3-tubulin

We evaluated the number of newborn cells after 5 days of BrdU administration in the 8th week after adipocyte-specific CB1 deletion (Fig. 3). We found a genotype effect on the number of BrdU-ir cells in the SGZ of DG ($F_{1,22} = 9.41$, $P < 0.01$). HFD-fed Ati-CB1-WT mice showed a decrease in the number of BrdU-ir cells as compared to SD-fed Ati-CB1-WT mice, while adipocyte-CB1 deficiency increased the number of BrdU-ir cells in a diet-independent manner (Fig. 3A). In the hypothalamus, diet effect on the number of BrdU-ir cells ($F_{1,22} = 6.73$, $P < 0.05$) is produced by an increase in the number of BrdU-ir cells in the ARC of HFD-fed Ati-CB1-WT mice and HFD-fed Ati-CB1-KO mice (Fig. 3B).

We also measured those BrdU-ir cells expressing β3-tubulin, a neuron-specific marker, indicating those newly born cells differentiated

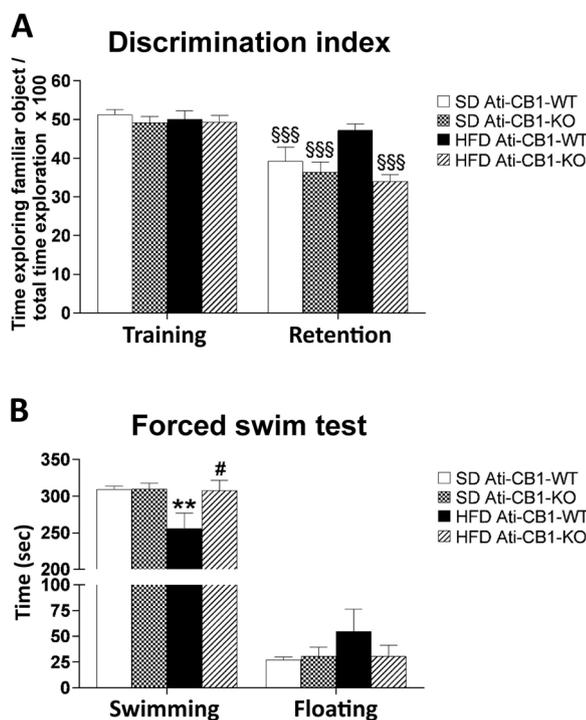


Fig. 2. Mice with adipocyte-specific CB1 deletion during DIO were tested for memory and depressive-like behavior in the NOR test (A) and the FS test (B) respectively. The histograms represent the mean ± SEM (n = 5–9). Sidak's test: SSS*P* < 0.001 vs. training; ***P* < 0.01 vs. SD-fed Ati-CB1-WT mice; #*P* < 0.05 vs. HFD-fed Ati-CB1-WT mice.

Table 1

Effects of high-fat diet and adipocyte-specific CB1 gene deletion on the mRNA expression of CB1, Caspase-3 and IL-6 in eWAT, skeletal muscle, hippocampus and/or hypothalamus^a.

		SD WT	SD KO	HFD WT	HFD KO	Interaction	Genotype	Diet
CB1	eWAT	1.00 ± 0.29	0.31 ± 0.04*	6.44 ± 2.37**	1.03 ± 0.27 [#]	ns	$F_{1,20} = 6.42$ $P = 0.019$	$F_{1,20} = 6.55$ $P = 0.018$
	Muscle	1.00 ± 0.05	0.74 ± 0.15	0.36 ± 0.03*	0.34 ± 0.05 [§]	ns	ns	$F_{1,19} = 16.43$ $P < 0.001$
	HC	1.00 ± 0.05	1.02 ± 0.10	1.15 ± 0.04*	1.13 ± 0.04	ns	ns	$F_{1,20} = 4.23$ $P = 0.05$
	HT	1.00 ± 0.08	0.84 ± 0.05	1.08 ± 0.06	0.81 ± 0.07 [#]	ns	$F_{1,20} = 9.39$ $P = 0.006$	ns
Caspase-3	HC	1.00 ± 0.06	0.99 ± 0.07	1.19 ± 0.02*	0.94 ± 0.06 [#]	$F_{1,20} = 4.18$ $P = 0.05$	$F_{1,20} = 4.21$ $P = 0.05$	ns
	HT	1.00 ± 0.04	0.98 ± 0.05	1.13 ± 0.05	0.97 ± 0.06	ns	ns	ns
IL-6	HC	1.00 ± 0.16	1.37 ± 0.09	1.47 ± 0.12*	0.89 ± 0.21 [#]	$F_{1,20} = 9.29$ $P = 0.006$	ns	ns
	HT	1.00 ± 0.14	0.95 ± 0.05	0.81 ± 0.09	0.43 ± 0.11 [#]	ns	ns	$F_{1,20} = 10.65$ $P = 0.003$

Two-way ANOVA and Bonferroni analysis (in case of interaction or main effect): ***/** $P < 0.05/0.01$ vs. SD-fed Ati-CB1-WT mice; [§] $P < 0.05$ vs. SD-fed Ati-CB1-KO mice; [#]/^{##} $P < 0.05/0.01$ vs. HFD-fed Ati-CB1-WT mice. Abbreviation: HC, hippocampus; HT, hypothalamus.

^a Values represent the mean ± SEM ($n = 6$).

into neurons (Fig. 3C, D). We only detected a genotype effect on the rate of BrdU-ir cells expressing β -tubulin in the SGZ ($F_{1,22} = 13.69$, $P < 0.01$), but not in ARC. This effect is produced by an increase in the rate of BrdU⁺/ β -tubulin⁺ cells (relative to total BrdU + cells) in the SGZ of HFD-fed Ati-CB1-KO mice (Fig. 3C). It should be noted that a low rate of newborn neurons (ranging 19.4–40.5% of the total BrdU⁺ cells) was found in the hypothalamus (Fig. 3D).

3.6. Cell differentiation and neuronal maturation in the hippocampus and hypothalamus: Bdnf and NeuN

Regarding the hippocampus, we found a diet effect on the Bdnf mRNA expression ($F_{1,20} = 5.14$, $P < 0.05$), with HFD-fed Ati-CB1-KO mice showing an increased expression (Fig. 3E). A genotype effect on NeuN mRNA expression in hippocampus ($F_{1,20} = 8.94$, $P < 0.01$) is produced by a decreased expression in HFD-fed Ati-CB1-WT mice and an increased expression in HFD-fed Ati-CB1-KO mice (Fig. 3E). Regarding the hypothalamus, interaction (diet and genotype) was specifically observed in the mRNA expression of Bdnf ($F_{1,20} = 8.94$, $P < 0.01$) and NeuN ($F_{1,20} = 22.65$, $P < 0.001$). A genotype effect was also found in the NeuN mRNA expression in the hypothalamus ($F_{1,20} = 22.65$, $P < 0.001$). Specifically, HFD increased mRNA expression of Bdnf and NeuN in the hypothalamus of Ati-CB1-WT mice (Fig. 3F). The adipocyte-specific CB1 deletion was sufficient to reverse the mRNA expression changes associated with HFD. Thus, HFD-fed Ati-CB1-KO mice had a decrease in hypothalamic mRNA expression of Bdnf and NeuN (Fig. 3F).

NeuN immunohistochemistry partially confirmed mRNA expression data. We found a diet effect on NeuN immunoreactivity ($F_{3,44} = 3.14$, $P < 0.03$) when DG and CA1 were analyzed separately. In particular, Bonferroni analysis indicated a decreased NeuN immunoreactivity in the hippocampal CA1 of HFD-fed Ati-CB1-WT mice, which was reversed in HFD-fed Ati-CB1-KO mice (Fig. 3G, H). In the hypothalamus, there was also a diet effect on NeuN immunoreactivity when the whole hypothalamus was analyzed ($F_{1,22} = 6.32$, $P < 0.02$), and specifically in ARC ($F_{1,22} = 6.48$, $P < 0.019$), where HFD-fed Ati-CB1-WT mice showed an increased immunolabeling compared to SD-fed Ati-CB1-WT mice. This significance was almost blunted in HFD-fed Ati-CB1-KO mice (Fig. 3I, J).

3.7. Effect of adipocyte-specific CB1 deletion on astrogliosis in the hippocampus and hypothalamus

Astrocytes, as determined by GFAP mRNA and protein expression,

was assessed in the hippocampus and hypothalamus of Ati-CB1-WT and Ati-CB1-KO mice in SD and HFD (Fig. 4). Interaction between diet and genotype was detected in the hippocampal GFAP mRNA expression ($F_{1,20} = 5.81$, $P = 0.025$). Thus, HFD increased hippocampal GFAP mRNA expression ($*P < 0.05$), which was reversed to control levels in HFD-fed Ati-CB1-KO mice ($^{\#}P < 0.05$) (Fig. 4A). When we evaluated the three hippocampal areas together, a genotype effect was found ($F_{1,66} = 4.48$, $P = 0.037$). We found a decrease in GFAP immunoreactivity in the hippocampus, in particular in the DG and CA1 of HFD-fed Ati-CB1-KO mice as compared to HFD-fed controls ($^{\#}P < 0.05$) (Fig. 4B, C). Conversely, we did not observe any main effect on GFAP expression in hypothalamus (Fig. 4D–F).

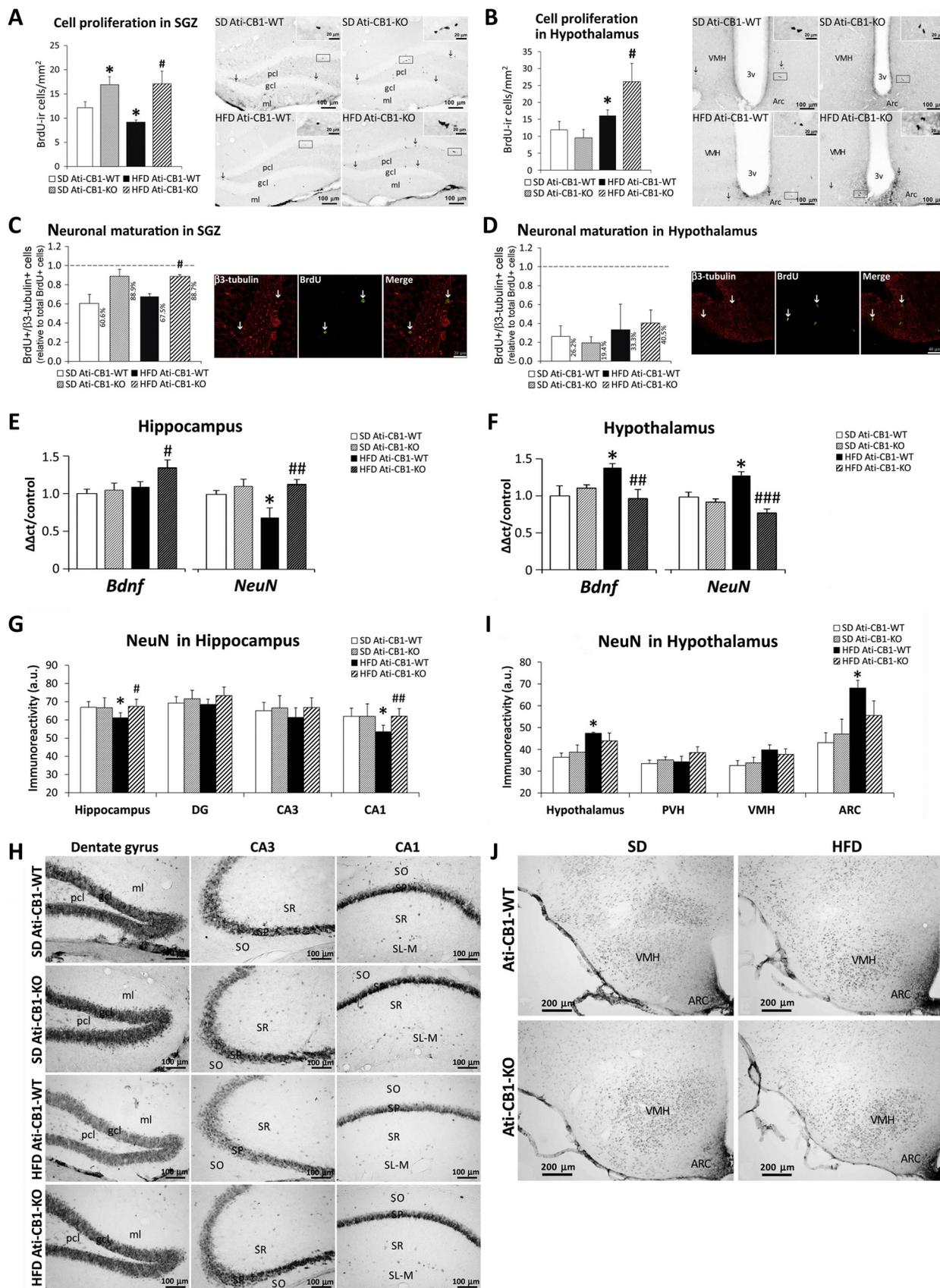
3.8. Effect of adipocyte-specific CB1 deletion on microgliosis in the hippocampus and hypothalamus

Microglia cells were also assessed by Iba-1 expression (Fig. 4). In the hippocampus, we observed a genotype effect on hippocampal Iba-1 mRNA expression ($F_{1,20} = 4.16$, $P = 0.05$) and Iba-1-ir cell number in all hippocampal areas ($F_{1,22} < 7.86$, $P < 0.01$). In particular, the adipocyte-specific CB1 deletion decreased Iba-1 mRNA expression in the hippocampus of HFD treated mice (Fig. 4G). This result was confirmed by immunohistochemical analysis of Iba1+ cells in all hippocampal regions (Fig. 4H–I); similar reductions of Iba1+ cell number have been found in Ati-CB1-KO mice compared to their respective controls. In the hypothalamus, a diet effect ($F_{1,22} = 5.77$, $P = 0.025$) was associated with an increase in the number of Iba-1-ir cells in the PVH of HFD-fed Ati-CB1-WT mice (Fig. 4J–L). Importantly, adipocyte-specific CB1 deletion was sufficient to revert this increase in PVH (Fig. 4K).

4. Discussion

In this study, we examined the hypothesis whether the adipocyte-specific CB1 deletion in obese mice, which were reported to exhibit metabolic improvements (Ruiz de Azua et al., 2017), also facilitates an amelioration of obesity-induced short-term memory deficits, depressive-like behaviors, and cellular remodeling in the brain. In fact, we found that metabolic improvements were accompanied by behavioral normalizations, increased adult neurogenesis and decreased inflammation in hippocampus and hypothalamus (Fig. 5).

Obese individuals have a higher prevalence of depression (Hryhorczuk et al., 2013; Capuron et al., 2017), anxiety (Garipey et al., 2010) and mild cognitive impairments (Dye et al., 2017; Solas et al., 2017). Conversely, depression can contribute to metabolic disorders,



(caption on next page)

Fig. 3. Effect of high-fat diet and adipocyte-specific CB1 deletion on cell proliferation and neuronal maturation in the subgranular zone (SGZ) of dentate gyrus and hypothalamus of mice fed SD and HFD by BrdU immunohistochemistry (A, B), double BrdU/ β -tubulin immunofluorescence (C, D), Bdnf and NeuN mRNA expression (E, F), and NeuN immunohistochemical expression (G–J). Representative micrographs show low- and high- (insets) magnification views of BrdU immunoreactivity and double immunofluorescence of the neuron-specific β -tubulin (green) and BrdU (red) of the typical clustering of newborn cells in the inner border of the granular cell layer and the arcuate nucleus (arrows), as well as immunohistochemical expression of the mature neuron-specific NeuN in the principal cell layers of the hippocampus and the main regions of the hypothalamus. The histograms represent the mean \pm SEM ($n = 6–8$). Bonferroni's test: * $P < 0.05$ vs. SD-fed Ati-CB1-WT mice; ###/###* $P < 0.05/0.01/0.001$ vs. HFD-fed Ati-CB1-WT mice (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

and correlates to weight gain and obesity (Capuron et al., 2017). Previously, we demonstrated that Ati-CB1-KO mice reversed anxiety-like behavior observed in obese mice (Ruiz de Azua et al., 2017). Therefore, we have further characterized the effect of the genetic inactivation of adipocyte CB1 in obesity-induced cognitive deficit and depressive-like behavior, using different behavioral paradigms. Obese mice showed a significant short-term memory impairment in the NOR test. Besides, these mice had a depressive-like behavior during FS test. These two behavioral phenotypes linked to obesity were reversed in HFD-fed Ati-CB1-KO mice. Therefore, adipocyte-specific CB1 signaling blockade had a profound impact in the alleviation of cognitive deficit and depressive-like behavior associated with obese mice.

The improvement in cognition and emotion in Ati-CB1-KO mice was linked to a metabolic amelioration, as reported previously (Ruiz de Azua et al., 2017). These results are in agreement with the improved metabolic functions in obese subjects treated with CB1 receptor antagonists (Scheen, 2008; Tam et al., 2012; Vida et al., 2014) and the resistance of total CB1-KO mice to develop DIO (Cota et al., 2003). In our previous study, the metabolic improvement observed in Ati-CB1-KO mice was consistent with decreases in the plasma levels of leptin and the inflammatory factors IL-6 and PAI1 (Ruiz de Azua et al., 2017).

Therefore, we further analyzed whether the beneficial effect of adipocyte CB1 blockade on metabolism and behavior was accompanied by a cellular brain remodeling in neuroinflammation and/or neurogenesis. However, we cannot exclude other potential mechanisms, for example, cell senescence which has recently been reported to facilitate obesity-induced anxiety (Ogrodnik et al., 2019). Obesity is also associated with a neuroinflammatory state that contributes to neurotoxicity, neuronal loss and glial activation (Teng et al., 2005; Posey et al., 2009; Ransohoff and Perry, 2009; Hidalgo et al., 2010). Obesity-associated brain inflammation has been linked to decreased spatial memory, anxiety and depression (Boitard et al., 2014; Duthiel et al., 2016; Guillemot-Legrís and Muccioli, 2017). Nonetheless, cognitive deficit in obesity can also occur in the absence of neuroinflammation (Guillemot-Legrís and Muccioli, 2017). In this manner, increased hippocampal CB1 gene expression concomitantly with an overactive endocannabinoid tone in obese mice could also contribute to impaired memory deficit, as suggested by other studies (Wise et al., 2009; Han et al., 2012). Obesity-derived neuroinflammation has been well-described in hypothalamus (Jais and Brüning, 2017) and other brain structures including hippocampus (Guillemot-Legrís and Muccioli, 2017). Accordingly, we found that obese mice showed increased expression of pro-inflammatory (hippocampal IL-6 mRNA), apoptotic (hippocampal caspase-3 mRNA), astroglial (hippocampal GFAP mRNA), and microglial (Iba-1-ir in PVN) markers. In hippocampus of obese mice, activated microglial could be the main responsible of the elevated IL-6 mRNA expression as suggested by previous findings (Valdearcos et al., 2014), although we cannot exclude a potential contribution of other cell types (e.g. endothelial cells) since Iba-1 expression was not modified in these mice as compared to SD-fed Ati-CB1-WT mice. Remarkably, microglial Iba-1 expression was enhanced locally in the PVH, a brain area largely involved in obesity and hyperphagia. Regarding genotype effects, HFD-fed Ati-CB1-KO mice had an overall reduction in the expression of the pro-inflammatory cytokine IL-6, consistent with a decrease in gliosis markers (GFAP, Iba-1) in hippocampus, while the hypothalamus showed a reduced IL-6 mRNA expression that could be consistent with a lower

Iba-1-ir cell number in PVH. In summary, adipocyte-specific CB1 deficiency is associated with an improvement of memory deficit, depressive-related disorders and neuroinflammation as compared to obese mice.

Furthermore, a single day of HFD is sufficient to increase IL-6, TNF- α and IL-1 β expression as well as microglial cell activation in the hypothalamus, even before body weight changes (Thaler et al., 2012). This suggests that hypothalamic inflammation may participate in the onset of weight gain, maybe through central insulin and leptin resistance (Posey et al., 2009; Hidalgo et al., 2010). Therefore, we put forward the notion that the reduced neuroinflammation in Ati-CB1-KO mice might be responsible of improved energy balance, and not only as a consequence of it.

Finally, we evaluated a potential link between improved cognitive and mood-related behavior and neurogenesis in obese Ati-CB1-KO mice. Several evidences have suggested that disrupted adult hippocampal neurogenesis may result in memory deficit (Deng et al., 2010) and depression (Eisch and Petrik, 2012). In this study, HFD-induced obesity also had a strong impact on cell proliferation in the hippocampus and hypothalamus, but in an opposite manner. In the hippocampus of obese mice, there was a decline in SGZ cell proliferation and an increase in cell death (apoptotic state assessed by increased caspase-3 expression) that could result in a lower number of mature neurons (NeuN), confirming previous studies (Lindqvist et al., 2006; Farr et al., 2008; Moraes et al., 2009). In contrast, the hypothalamus of obese mice had an increase in cell proliferation (BrdU) and mature neurons (NeuN), and no effect on cell death (caspase-3) or on the rate of newborn neurons (percentage of BrdU⁺/ β -tubulin + cells with respect to total BrdU⁺ cells), suggesting an imbalance of hypothalamic neuronal turnover. Further studies should clarify the underlying mechanism of the increased neuronal survival (NeuN). Remarkably, NeuN + cells were increased in ARC, a metabolically relevant hypothalamic region, where most BrdU-ir cells were found. It has been shown that obesity inhibits adult hypothalamic neurogenesis (McNay et al., 2012) and induces neuronal cell death (Moraes et al., 2009; Thaler et al., 2012). Interestingly, hypothalamic neurogenesis contributes to food intake regulation in response to leptin and fasting conditions (Pierce and Xu, 2010; Lee et al., 2012). Here, adipocyte-specific CB1 deletion was linked to an increased cell proliferation rate in both neurogenic niches, SGZ and ARC. However, exclusively in the hippocampus, the increased cell proliferation was associated with higher Bdnf and NeuN mRNA expression (survival) and higher number of BrdU-ir cells expressing β -tubulin in the SGZ (neuronal maturation) in HFD-fed Ati-CB1-KO mice as compared to controls. Accordingly, pharmacological blockade of CB1 specifically increased cell proliferation in the SGZ of HFD-fed rats, but not in SD-fed rats (Rivera et al., 2011). These results suggest that increased hippocampal neurogenesis was associated with CB1-induced alleviation of energy imbalance. In agreement, dietary restriction significantly decreases the hippocampal levels of AEA and 2-AG (Rivera et al., 2013), and induced neural proliferation and BDNF expression in the DG (Lee et al., 2000; Kumar et al., 2009). However, contradictory evidence was related to neurogenesis in total CB1-KO mice (Wolf et al., 2010).

In conclusion, the present study demonstrates that adipocyte-specific CB1 deficiency alleviates obesity-related memory deficits, depressive-like behavior, adult neurogenesis and neuroinflammation.

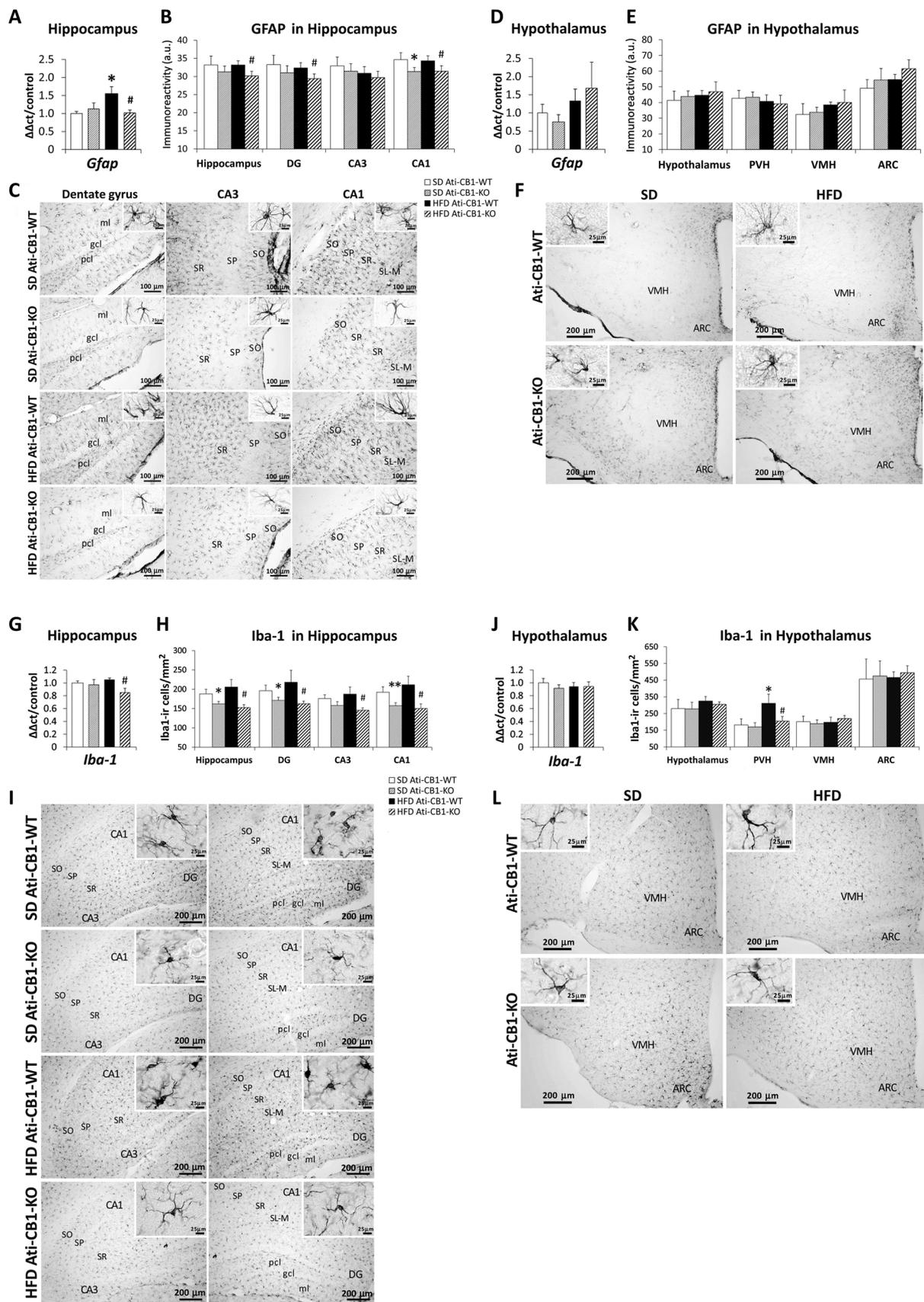


Fig. 4. Effect of high-fat diet and induction of adipocyte-specific CB1 deletion on gliosis in the hippocampus and the hypothalamus of mice fed SD and HFD on *Gfap* mRNA expression and GFAP immunohistochemistry (A-F), and *Iba-1* mRNA expression and *Iba-1* immunohistochemistry (G-L). Representative micrographs show low- and high- (insets) magnification views of GFAP and *Iba-1* immunoreactivity in the hippocampus and the hypothalamus (C, F, I, L). The histograms represent the mean \pm SEM ($n = 6-8$). Bonferroni's test: */** $P < 0.05/0.01$ vs. SD-fed Ati-CB1-WT mice; # $P < 0.05$ vs. HFD-fed Ati-CB1-WT mice.

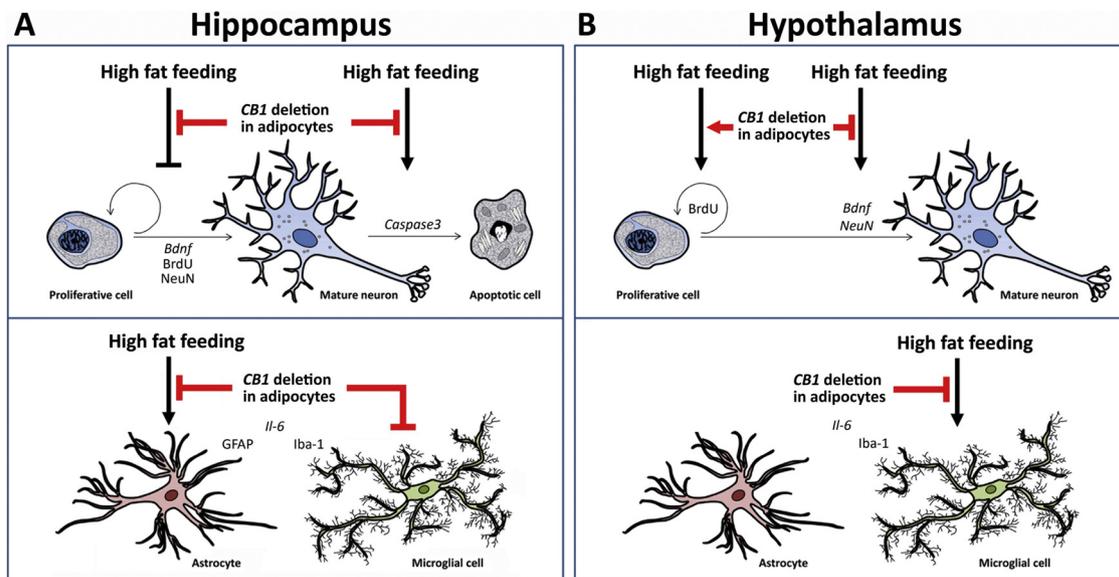


Fig. 5. Simplified scheme depicting the main effects of high-fat feeding and conditional adipocyte-specific CB1 gene deletion on cell proliferation (Bdnf, BrdU), maturation (NeuN), pro-apoptosis (Caspase 3), inflammation (IL-6) or gliosis (presence of astroglial GFAP, and microglial Iba-1) in the hippocampus (A) and/or the hypothalamus (B).

These results support the action of an adipocyte-brain crosstalk whereby adipocyte CB1 plays a critical role in brain remodeling. Future studies should identify the molecular mechanisms that link how deficiency of adipocyte CB1 leads to normal brain homeostasis despite high-fat feeding.

Author contributions

JS, FRF, IRA and BL were responsible for concept and design of the study. JS, PR, AAR MPM, SA and IRA contributed to data acquisition and analysis. JS, FRF, IRA and BL contributed to data interpretation. All authors were involved in drafting the manuscript and final approval of the version to be published.

Funding

This work was supported by 7th Framework Programme of European Union [HEALTH-F2-2008-223713, REPROBESITY]; Instituto de Salud Carlos III (ISCIII), Ministerio de Economía y Competitividad (MINECO) co-funded by ERDF-EU [JS: CP12/03109, PI16/01374; FRF: PI16/01698]; Consejería de Economía, Innovación, Ciencia y Empleo, Junta de Andalucía, ERDF-EU [FRF: CTS-8221]; Consejería de Salud, Junta de Andalucía, ERDF-EU (FRF: SAS111224); JS holds a “Miguel Servet II” research contract from the National System of Health, ISCIII, ERDF-EU [CPII17/00024]. PR holds a “Sara Borrell” research contract from the National System of Health, ISCIII, ERDF-EU [CD16/00067].

Declaration of Competing Interest

None.

Acknowledgements

The authors thank to Giacomo Mancini for excellent assistance in establishing the experimental genetic model. Support by the IMB Microscopy Core Facility (Mainz, Germany) is gratefully acknowledged.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.psyneuen.2019.104418>.

104418.

References

- André, C., Guzman-Quevedo, O., Rey, C., Rémus-Borel, J., Clark, S., Castellanos-Jankiewicz, A., Ladeveze, E., Leste-Lasserre, T., Nadjar, A., Arous, D.N., Laye, S., Cota, D., 2017. Inhibiting microglia expansion prevents diet-induced hypothalamic and peripheral inflammation. *Diabetes* 66, 908–919. <https://doi.org/10.2337/db16-0586>.
- Boitard, C., Cavaroc, A., Sauvart, J., Aubert, A., Castanon, N., Laye, S., Ferreira, G., 2014. Impairment of hippocampal-dependent memory induced by juvenile high-fat diet intake is associated with enhanced hippocampal inflammation in rats. *Brain Behav. Immun.* 40, 9–17. <https://doi.org/10.1016/j.bbi.2014.03.005>.
- Buckman, L.B., Thompson, M.M., Lippert, R.N., Blackwell, T.S., Yull, F.E., Ellacott, K.L., 2014. Evidence for a novel functional role of astrocytes in the acute homeostatic response to high-fat diet intake in mice. *Mol. Metab.* 4, 58–63. <https://doi.org/10.1016/j.molmet.2014.10.001>.
- Capuron, L., Lasselín, J., Castanon, N., 2017. Role of adiposity-driven inflammation in depressive morbidity. *Neuropsychopharmacol. Rev.* 42, 115–128.
- Cluny, N.L., Reimer, R.A., Sharkey, K.A., 2012. Cannabinoid signaling regulates inflammation and energy balance: the importance of the brain-gut axis. *Brain Behav. Immun.* 26, 691–698. <https://doi.org/10.1016/j.bbi.2012.01.004>.
- Cota, D., Marsicano, G., Tschöp, M., Grübler, Y., Flachskamm, C., Schubert, M., Auer, D., Yassouridis, A., Thöne-Reineke, C., Ortman, S., Tomassoni, F., Cervino, C., Nisoli, E., Linthorst, A.C., Pasquali, R., Lutz, B., Stalla, G.K., Pagotto, U., 2003. The endogenous cannabinoid system affects energy balance via central orexinergic drive and peripheral lipogenesis. *J. Clin. Invest.* 112, 423–431.
- Deng, W., Aimone, J.B., Gage, F.H., 2010. New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? *Nat. Rev. Neurosci.* 11, 339–350. <https://doi.org/10.1038/nrn2822>.
- Donzis, E.J., Tronson, N.C., 2014. Modulation of learning and memory by cytokines: signaling mechanisms and long term consequences. *Neurobiol. Learn. Mem.* 115, 68–77. <https://doi.org/10.1016/j.nlm.2014.08.008>.
- Dutheil, S., Ota, K.T., Wohleb, E.S., Rasmussen, K., Duman, R.S., 2016. High-fat diet induced anxiety and anhedonia: impact on brain homeostasis and inflammation. *Neuropsychopharmacology* 41, 1874–1887.
- Dye, L., Boyle, N.B., Champ, C., Lawton, C., 2017. The relationship between obesity and cognitive health and decline. *Proc. Nutr. Soc.* 76, 443–454.
- Eisch, A.J., Petrik, D., 2012. Depression and hippocampal neurogenesis: a road to remission? *Science* 338, 72–75. <https://doi.org/10.1126/science.1222941>.
- Farr, S.A., Yamada, K.A., Butterfield, D.A., Abdul, H.M., Xu, L., Miller, N.E., et al., 2008. Obesity and hypertriglyceridemia produce cognitive impairment. *Endocrinology* 149, 2628–2636. <https://doi.org/10.1210/en.2007-1722>.
- García-Ovejero, D., Arévalo-Martín, Á., Navarro-Galve, B., Pinteaux, E., Molina-Holgado, E., Molina-Holgado, F., 2013. Neuroimmune interactions of cannabinoids in neurogenesis: focus on interleukin-1 β (IL-1 β) signalling. *Biochem. Soc. Trans.* 41, 1577–1582. <https://doi.org/10.1042/BST20130198>.
- Gariépy, G., Nitka, D., Schmitz, N., 2010. The association between obesity and anxiety disorders in the population: a systemic review and meta-analysis. *Int. J. Obes.* 34, 407–419. <https://doi.org/10.1038/ijo.2009.252>.
- Guillemot-Legrís, O., Muccioli, G.G., 2017. Obesity-induced neuroinflammation: beyond the hypothalamus. *Trends Neurosci.* 40, 237–253. <https://doi.org/10.1016/j.tins.2017.02.005>.
- Han, J., Kesner, P., Metna-Laurent, M., Duan, T., Xu, L., Georges, F., Koehl, M., Arous, D.

- D.N., Mendizabal-Zubiaga, J., Grandes, P., Liu, Q., Bai, G., Wang, W., Xiong, L., Ren, W., Marsicano, G., Zhang, X., 2012. Acute cannabinoids impair working memory through astroglial CB1 receptor modulation of hippocampal LTD. *Cell* 148, 1039–1050. <https://doi.org/10.1016/j.cell.2012.01.037>.
- Hidalgo, J., Florit, S., Giralt, M., Ferrer, B., Keller, C., Pilegaard, H., 2010. Transgenic mice with astrocyte-targeted production of interleukin-6 are resistant to high-fat diet-induced increases in body weight and body fat. *Brain Behav. Immun.* 24, 119–126. <https://doi.org/10.1016/j.bbi.2009.09.002>.
- Higuchi, S., Irie, K., Mishima, S., Araki, M., Ohji, M., Shirakawa, A., Akitake, Y., Matsuyama, K., Mishima, K., Mishima, K., Iwasaki, K., Fujiwara, M., 2010. The cannabinoid 1-receptor silent antagonist O-2050 attenuates preference for high-fat diet and activated astrocytes in mice. *J. Pharmacol. Sci.* 112, 369–372. <https://doi.org/10.1254/jphs.09326SC>.
- Hryhorczuk, C., Sharma, S., Fulton, S.E., 2013. Metabolic disturbance connecting obesity and depression. *Front. Neurosci.* 7, 1. <https://doi.org/10.3389/fnins.2013.00177>.
- Jais, A., Brüning, J.C., 2017. Hypothalamic inflammation in obesity and metabolic disease. *J. Clin. Invest.* 127, 24–32. <https://doi.org/10.1172/JCI88878>.
- Kumar, S., Parkash, J., Kataria, H., Kaur, G., 2009. Interactive effect of excitotoxic injury and dietary restriction on neurogenesis and neurotrophic factors in adult male rat brain. *Neurosci. Res.* 65, 367–374. <https://doi.org/10.1016/j.neures.2009.08.015>.
- Lambertsen, K.L., Clausen, B.H., Babcock, A.A., Gregersen, R., Fenger, C., Nielsen, H.H., Haugaard, L.S., Wirenfeldt, M., Nielsen, M., Dagnaes-Hansen, F., Blüthmann, H., Faergeman, N.J., Meldgaard, M., Deierborg, T., Finsen, B., 2009. Microglia protect neurons against ischemia by synthesis of tumor necrosis factor. *J. Neurosci.* 29, 1319–1330. <https://doi.org/10.1523/JNEUROSCI.5505-08.2009>.
- Lasselain, J., Capuron, L., 2014. Chronic low-grade inflammation in metabolic disorders: relevance for behavioral symptoms. *Neuroimmunomodulation* 21, 95–101. <https://doi.org/10.1159/000356535>.
- Lee, D.A., Bedont, J.L., Pak, T., Wang, H., Song, J., Miranda-Angulo, A., Takiar, V., Charubhumi, V., Balordi, F., Takebayashi, H., Aja, S., Ford, E., Fishell, G., Blackshaw, S., 2012. Tanycytes of the hypothalamic median eminence form a diet-responsive neurogenic niche. *Nat. Neurosci.* 15, 700–702. <https://doi.org/10.1038/nn.3079>.
- Lee, J., Duan, W., Long, J.M., Ingram, D.K., Mattson, M.P., 2000. Dietary restriction increases the number of newly generated neural cells, and induces BDNF expression, in the dentate gyrus of rats. *J. Mol. Neurosci.* 15, 99–108. <https://doi.org/10.1385/JMN:15:2:99>.
- Lindqvist, A., Mohapel, P., Bouter, B., Frielingsdorf, H., Pizzo, D., Brundin, P., Erlanson-Albertsson, C., 2006. High-fat diet impairs hippocampal neurogenesis in male rats. *Eur. J. Neurosci.* 13, 1385–1388. <https://doi.org/10.1111/j.1468-1331.2006.01500.x>.
- Lumeng, C.N., Bodzin, J.L., Saltiel, A.R., 2007. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J. Clin. Invest.* 117, 175–184. <https://doi.org/10.1172/JCI29881>.
- Maccarrone, M., Guzmán, M., Mackie, K., Doherty, P., Harkany, T., 2014. Programming of neural cells by (endo)cannabinoids: from physiological rules to emerging therapies. *Nat. Rev. Neurosci.* 15, 786–801. <https://doi.org/10.1038/nrn3846>.
- Malcher-Lopes, R., Di, S., Marcheselli, V.S., Weng, F.J., Stuart, C.T., Bazan, N.G., Tasker, J.G., 2006. Opposing crosstalk between leptin and glucocorticoids rapidly modulates synaptic excitation via endocannabinoid release. *J. Neurosci.* 26, 6643–6650. <https://doi.org/10.1523/JNEUROSCI.5126-05.2006>.
- McNay, D.E., Briancion, N., Kokoeva, M.V., Maratos-Flier, E., Flier, J.S., 2012. Remodeling of the arcuate nucleus energy-balance circuit is inhibited in obese mice. *J. Clin. Invest.* 122, 142–152. <https://doi.org/10.1172/JCI43134>.
- Metna-Laurent, M., Marsicano, G., 2015. Rising stars: modulation of brain functions by astroglial type-1 cannabinoid receptors. *Glia* 63, 353–364. <https://doi.org/10.1002/glia.22773>.
- Moraes, J.C., Coope, A., Morari, J., Cintra, D.E., Roman, E.A., Pauli, J.R., Romanatto, T., Carvalho, J.B., Oliveira, A.L., Saad, M.J., Velloso, L.A., 2009. High-fat diet induces apoptosis of hypothalamic neurons. *PLoS One* 4, e5045. <https://doi.org/10.1371/journal.pone.0005045>.
- Nakanishi, M., Niidome, T., Matsuda, S., Akaike, A., Kihara, T., Sugimoto, H., 2007. Microglia-derived interleukin-6 and leukemia-inhibitory factor promote astrocytic differentiation of neural stem/progenitor cells. *Eur. J. Neurosci.* 25, 649–658. <https://doi.org/10.1111/j.1460-9568.2007.05309.x>.
- Ogrodnik, M., Zhu, Y., Langhi, L.G.P., Tchkonina, T., Krüger, P., Fielder, E., Victorelli, S., Ruswhandi, R.A., Giorgadze, N., Pirtskhalava, T., Podgorni, O., Enikolopov, G., Johnson, K.O., Xu, M., Inman, C., Palmer, A.K., Schafer, M., Weigl, M., Ikeno, Y., Burns, T.C., Passos, J.F., von Zglinicki, T., Kirkland, J.L., Jurk, D., 2019. Obesity-induced cellular senescence drives anxiety and impairs neurogenesis. *Cell Metab.* 29, 1–17. <https://doi.org/10.1016/j.cmet.2018.12.008>.
- Ortega-Gutiérrez, S., Molina-Holgado, E., Guaza, C., 2005. Effect of anandamide uptake inhibition in the production of nitric oxide and in the release of cytokines in astrocyte cultures. *Glia* 52, 163–168. <https://doi.org/10.1002/glia.20229>.
- Osei-Hyiaman, D., Harvey-White, J., Bátkai, S., Kunes, G., 2006. The role of the endocannabinoid system in the control of energy homeostasis. *Int. J. Obes. (Lond.)* 30 (Suppl 1), S33–S8. <https://doi.org/10.1038/sj.ijo.0803276>.
- Pagotto, U., Marsicano, G., Cota, D., Lutz, B., Pasquali, R., 2006. The emerging role of the endocannabinoid system in endocrine regulation and energy balance. *Endocr. Rev.* 27, 73–100. <https://doi.org/10.1210/er.2005-0009>.
- Pierce, A.A., Xu, A.W., 2010. De novo neurogenesis in adult hypothalamus as a compensatory mechanism to regulate energy balance. *J. Neurosci.* 30, 723–730. <https://doi.org/10.1523/JNEUROSCI.2479-09.2010>.
- Posey, K.A., Clegg, D.J., Printz, R.L., Byun, J., Morton, G.J., Vivekanandan-Giri, A., Pennathur, S., Baskin, D.G., Heinecke, J.W., Woods, S.C., Schwartz, M.W., Niswender, K.D., 2009. Hypothalamic proinflammatory lipid accumulation, inflammation, and insulin resistance in rats fed a high-fat diet. *Am. J. Physiol. Endocrinol. Metab.* 296, E1003–12. <https://doi.org/10.1152/ajpendo.90377.2008>.
- Purkayastha, S., Cai, D., 2013a. Disruption of neurogenesis by hypothalamic inflammation in obesity or aging. *Rev. Endocr. Metab. Disord.* 14, 351–356. <https://doi.org/10.1007/s11154-013-9279-z>.
- Purkayastha, S., Cai, D., 2013b. Neuroinflammatory basis of metabolic syndrome. *Mol. Metab.* 2, 356–363. <https://doi.org/10.1016/j.molmet.2013.09.005>.
- Quarta, C., Bellocchio, L., Mancini, G., Mazza, R., Cervino, C., Braulke, L.J., Fekete, C., Latorre, R., Nanni, C., Bucci, M., Clemens, L.E., Heldmaier, G., Watanabe, M., Leste-Lassere, T., Maitre, M., Tedesco, L., Fanelli, F., Reuss, S., Klaus, S., Srivastava, R.K., Monory, K., Valerio, A., Grandis, A., De Giorgio, R., Pasquali, R., Nisoli, E., Cota, D., Lutz, B., Marsicano, G., Pagotto, U., 2010. CB(1) signaling in forebrain and sympathetic neurons is a key determinant of endocannabinoid actions on energy balance. *Cell Metab.* 11, 273–285. <https://doi.org/10.1016/j.cmet.2010.02.015>.
- Ransohoff, R.M., Perry, V.H., 2009. Microglial physiology: unique stimuli, specialized responses. *Annu. Rev. Immunol.* 27, 119–145. <https://doi.org/10.1146/annurev.immunol.021908.132528>.
- Rivera, P., Luque-Rojas, M.J., Pastor, A., Blanco, E., Pavón, F.J., Serrano, A., Crespiello, A., Vida, M., Grondona, J.M., Cifuentes, M., Bermúdez-Silva, F.J., de la Torre, R., de Fonseca, F.R., Suárez, J., 2013. Diet-dependent modulation of hippocampal expression of endocannabinoid signaling-related proteins in cannabinoid antagonist-treated obese rats. *Eur. J. Neurosci.* 37, 105–117. <https://doi.org/10.1111/ejn.12012>.
- Rivera, P., Romero-Zerbo, Y., Pavón, F.J., Serrano, A., López-Avalos, M.D., Cifuentes, M., Grondona, J.M., Bermúdez-Silva, F.J., Fernández-Llebrez, P., de Fonseca, F.R., Suárez, J., Pérez-Martín, M., 2011. Obesity-dependent cannabinoid modulation of proliferation in adult neurogenic regions. *Eur. J. Neurosci.* 33, 1577–1586. <https://doi.org/10.1111/j.1460-9568.2011.07650.x>.
- Ruiz de Aza, I., Lutz, B., 2019. Multiple endocannabinoid-mediated mechanisms in the regulation of energy homeostasis in brain and peripheral tissues. *Cell. Mol. Life Sci.* 76, 1341–1363. <https://doi.org/10.1007/s00118-018-2994-6>.
- Ruiz de Aza, I., Mancini, G., Srivastava, R.K., Rey, A.A., Cardinal, P., Tedesco, L., Zingaretti, C.M., Sassmann, A., Quarta, C., Schwitter, C., Conrad, A., Wettschurek, N., Vemuri, V.K., Makriyannis, A., Hartwig, J., Mendez-Lago, M., Bindila, L., Monory, K., Giordano, A., Cinti, S., Marsicano, G., Offermanns, S., Nisoli, E., Pagotto, U., Cota, D., Lutz, B., 2017. Adipocyte cannabinoid receptor CB1 regulates energy homeostasis and alternatively activated macrophages. *J. Clin. Invest.* 127, 4148–4162. <https://doi.org/10.1172/JCI83626>.
- Scheen, A.J., 2008. CB1 receptor blockade and its impact on cardiometabolic risk factors: overview of the RIO programme with rimonabant. *J. Neuroendocrinol.* 20 (Suppl. 1), 139–146. <https://doi.org/10.1111/j.1365-2826.2008.01681.x>.
- Silvestri, C., Di Marzo, V., 2013. The endocannabinoid system in energy homeostasis and the etiology of metabolic disorders. *Cell Metab.* 17, 475–490. <https://doi.org/10.1016/j.cmet.2013.03.001>.
- Solas, M., Milagro, F.I., Ramirez, M.J., Martinez, J.A., 2017. Inflammation and gut-brain axis link obesity to cognitive dysfunction: plausible pharmacological interventions. *Current Opinion in Pharmacol* 37, 87–92. <https://doi.org/10.1016/j.coph.2017.10.005>.
- Stella, N., 2010. Cannabinoid and cannabinoid-like receptors in microglia, astrocytes, and astrocytomas. *Glia* 58, 1017–1030. <https://doi.org/10.1002/glia.20983>.
- Tam, J., Cinar, R., Liu, J., Godlewski, G., Wesley, D., Jourdan, T., Szanda, G., Mukhopadhyay, B., Chedester, L., Liow, J.S., Innis, R.B., Cheng, K., Rice, K.C., Deschamps, J.R., Chorvat, R.J., McElroy, J.F., Kuno, G., 2012. Peripheral cannabinoid-1 receptor inverse agonism reduces obesity by reversing leptin resistance. *Cell Metab.* 16, 167–179. <https://doi.org/10.1016/j.cmet.2012.07.002>.
- Teng, H.K., Teng, K.K., Lee, R., Wright, S., Tevar, S., Almeida, R.D., Kermani, P., Torkin, R., Chen, Z.Y., Lee, F.S., Kraemer, R.T., Nykjaer, A., Hempstead, B.L., 2005. ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. *J. Neurosci.* 25, 5455–5463. <https://doi.org/10.1523/JNEUROSCI.5123-04.2005>.
- Thaler, J.P., Yi, C.X., Schur, E.A., Guyenet, S.J., Hwang, B.H., Dietrich, M.O., Zhao, X., Sarruf, D.A., Izgur, V., Maravilla, K.R., Nguyen, H.T., Fischer, J.D., Matsen, M.E., Wisse, B.E., Morton, G.J., Horvath, T.L., Baskin, D.G., Tschöp, M.H., Schwartz, M.W., 2012. Obesity is associated with hypothalamic injury in rodents and humans. *J. Clin. Invest.* 122, 153–162. <https://doi.org/10.1172/JCI59660>.
- Valdearcos, M., Douglass, J.D., Robblee, M.M., Dorfman, M.D., Stifler, D.R., Bennett, M.L., Gerritsie, I., Fasnacht, R., Barres, B.A., Thaler, J.P., Koliwad, S.K., 2017. Microglial inflammatory signaling orchestrates the hypothalamic immune response to dietary excess and mediates obesity susceptibility. *Cell Metab.* 26, 185–197. <https://doi.org/10.1016/j.cmet.2017.05.015>.
- Valdearcos, M., Robblee, M.M., Benjamin, D.I., Nomura, D.K., Xu, A.W., Koliwad, S.K., 2014. Microglia dictate the impact of saturated fat consumption on hypothalamic inflammation and neuronal function. *Cell Rep.* 9, 2124–2138. <https://doi.org/10.1016/j.celrep.2014.11.018>.
- Vida, M., Rivera, P., Gavito, A.L., Suárez, J., Pavón, F.J., Arrabal, S., Romero-Cuevas, M., Bautista, D., Martínez, A., de Fonseca, F.R., Serrano, A., Baixeras, E., 2014. CB1 blockade potentiates down-regulation of lipogenic gene expression in perirenal adipose tissue in high carbohydrate diet-induced obesity. *PLoS One* 9, e90016. <https://doi.org/10.1371/journal.pone.0090016>.
- Wise, L.E., Thorpe, A.J., Lichtman, A.H., 2009. Hippocampal CB(1) receptors mediate the memory impairing effects of Delta(9)-tetrahydrocannabinol. *Neuropsychopharmacology* 34, 2072–2080. <https://doi.org/10.1038/npp.2009.31>.
- Wolf, S.A., Bick-Sander, A., Fabel, K., Leal-Galicia, P., Tauber, S., Ramirez-Rodriguez, G., Müller, A., Melnik, A., Waltinger, T.P., Ullrich, O., Kempermann, G., 2010. Cannabinoid receptor CB1 mediates baseline and activity-induced survival of new neurons in adult hippocampal neurogenesis. *Cell Commun. Signal* 8, 12. <https://doi.org/10.1186/1478-811X-8-12>.
- Zimmermann, T., Maroso, M., Beer, A., Baddenhausen, S., Ludewig, S., Fan, W., Vennin, C., Loch, S., Berninger, B., Hofmann, C., Korte, M., Soltesz, I., Lutz, B., Leschik, J., 2018. Neural stem cell lineage-specific cannabinoid type-1 receptor regulates neurogenesis and plasticity in the adult mouse hippocampus. *Cereb. Cortex* 28, 4454–4471. <https://doi.org/10.1093/cercor/bhy258>. In this issue.