Peroxisomal Proliferator-Activated Receptor β/δ Deficiency Induces Cognitive Alterations

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Research

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Abstract

Background

PPARβ/δ, the most PPAR abundant isotype in the central nervous system is involved in the modulation of microglial homeostasis and metabolism. Several studies have demonstrated that people suffering from type 2 diabetes mellitus develop cognitive decline turning insulin resistance one of the best predictors of this disturbance. Although numerous investigations have studied the role of PPARb/d in metabolism, its role in neuronal and cognitive function has been underexplored. Therefore, the aim of the study is to determine the role of PPARb/d in the neuropathological pathways involved in the development of cognitive decline and as to whether a risk factor involved in cognitive loss such as obesity modulates neuropathological markers.

6-month-old male PPARβ/δ-null (PPARβ/$\delta^{-/-}$) and wildtype (WT) littermates with the same genetic background (C57BL/6X129/SV) and exceptionally, C57BL/6 were used. After the weaning, animals were fed either with conventional chow (CT) or with a palmitic acid-enriched diet containing 45% of fat mainly from hydrogenated coconut oil (HFD). Thus, four groups were defined: WT CT, WT HFD, PPARβ/$\delta^{-/-}$ CT and PPARβ/$\delta^{-/-}$ HFD and several pathological mechanisms involved in cognitive decline were analyzed.

Results

Our results confirmed that C57BL/6X129/SV showed significantly increased levels of anxiety compared to C57BL/6. Therefore, to evaluate cognitive decline, behavioral tests were dismissed, and dendritic spine quantification and other biochemical biomarkers were performed.

PPARβ/$\delta^{-/-}$ mice exhibited a decrease in dendritic spine density and synaptic markers, suggesting an alteration in cognitive function and synaptic plasticity. Likewise, our study demonstrated that the lack of PPARβ/δ receptor enhances gliosis in the hippocampus, contributing to astrocyte and microglial activation and also induced an increase in neuroinflammatory biomarkers. Additionally, alterations in the hippocampal insulin receptor pathway were found. Interestingly, while some of the disturbances caused by the lack of PPARβ/δ were not affected by feeding the HFD, others were exacerbated or required the combination of both factors.

Conclusions

Taken together, these findings suggest that the loss of PPARβ/$\delta^{-/-}$ affects neuronal and synaptic structure, contributing to cognitive dysfunction and, they also present this receptor as a possible new target for the treatment of cognitive decline.

Background

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that belong to the nuclear receptor superfamily (1). PPARs are activated by natural ligands derived from dietary lipids, such as polyunsaturated fatty acids and their derivatives and exert an important physiological role in regulating glucose, lipid and lipoprotein metabolism. Likewise, these receptors can be also activated by synthetic ligands like fibrates, glitazones or nonsteroidal anti-inflammatory drugs (NSAIDs) (2, 3), which make them promising targets for several pathologies. Thus, the interest in the medical field for these drug targets has increased exponentially in the last years.

The PPAR subfamily comprises three isotypes: PPARα, PPARγ and PPARβ/δ. Several studies have shown that PPARα and PPARγ activation mediates by promoting the regulation of pathologic processes including neuroinflammation, mitochondrial alterations and cognitive decline (4, 5). Interestingly, although PPARβ/δ has been shown to be the most abundant isotype in the central nervous system (CNS) (6), being expressed in the main cellular components of this system including astrocytes, neurons and microglia (7), its role in neurodegenerative disorders has not been well characterized.

Inflammation not only actively contributes to the development of several neurodegenerative diseases including Alzheimer´s disease (AD) (8, 9), but also plays an essential role in the progression of metabolic pathologies, being a key point where both pathologies converge. In fact, there is multiple evidence that insulin resistance is one of the best predictors of cognitive decline, supporting the hypothesis that AD represents a form of diabetes mellitus that selectively affects the brain, receiving the name of “type 3 diabetes” (10). Moreover, several studies demonstrated that people suffering from type 2 diabetes mellitus (T2DM) also develop cognitive decline (11, 12). Notably, it has been shown that PPARβ/δ downregulation could be linked to both, neuroinflammation and insulin resistance in the brain (13). In fact, several clinical trials have suggested that PPARβ/δ activation reduces inflammation and ameliorates insulin sensitivity (14–16), among others. Therefore, they have been considered as good candidates for the treatment of these pathologies characterized by these hallmarks, such as T2DM (17). In the CNS, synthetic PPARβ/δ-specific agonists have been reported to ameliorate clinical symptoms, reducing the severity of a variety of CNS pathologies by modulating oxidative stress and inflammatory responses associated with these diseases (18, 19).

Collectively, although PPARβ/δ seems to play a key role in several pathologic processes, including cognitive decline, the mechanisms responsible for these effects remain unknown. Therefore, the aim of the study is to determine the role of PPARβ/δ in the neuropathological pathways involved in the development of cognitive decline and as to whether a risk factor involved in cognitive loss such as obesity (high fat diet, HFD) consumption) modulates hippocampal neuropathological markers in mice lacking this nuclear receptor.

Methods

2.1. Animals

To perform this study, 6-month-old PPARβ/δ-null (PPARβ/$\delta^{-/-}$)(20) and wild type (WT) littermates with the same genetic background (C57BL/6X129/SV) were used. Exceptionally, to perform open field test, C57BL/6 mice also were used. In all cases, animals were obtained from established breeding couples in the animal facility (Animal facility from the Pharmacy and Food Sciences Faculty from the University of Barcelona; approval number C-0032). After the weaning (at 21 days-old), and throughout their growth, animals were fed either with conventional chow (control diet, CT) (ENVIGO, Madison, Wt 53744-4220) or with a palmitic acid-enriched diet containing 45% of fat mainly from hydrogenated coconut oil (HFD) (Research Diets Inc., New Brunswick, USA). Thus, four groups were defined: WT CT, WT HFD, PPARβ/$\delta^{-/-}$ CT and PPARβ/$\delta^{-/-}$ HFD.

All animals were kept under stable conditions of humidity and temperature, standard light-dark cycle (12-h light/dark cycle) and food and water *ad libitum*, following the ethics guidelines defined by the European Committee (European Communities Council Directive 2010/63/EU). Manipulation protocols were previously approved by the ethics committee from the University of Barcelona and, at all times it was made sure that animal numbers, their stress and pain were kept under a necessary minimum following the appropriate animal manipulation ethical methodologies.

Glucose and insulin tolerance tests

For both tests, mice were fasted for 6 h and the tests were performed in a quiet room, preheated to +28 ºC. In the glucose tolerance test (GTT), glucose was administered at a dose of 1g/kg intraperitoneally (i.p). On the other hand, in the insulin tolerance test (ITT), the dosage of insulin used was 0.75 IU/Kg and it was also administered i.p. Next, samples from the tail vein were extracted in consecutive periods of time. In GTT, measurements were made at 5, 15, 30, 60 and 120 min after the administration of glucose. In the ITT case, samples were extracted at 15, 30, 45, 60 and 90 min after the insulin administration.

The animals were monitored in every moment, and in those cases where glucose levels dropped under a concentration of 20 mg/dL, a dosage of 1 mg/Kg of glucose was administered i.p. and they were kept in observation until blood glucose concentrations were stable and the animal behavior was normal. Twelve animals per group were analyzed.

Open field behavior

The open field behavioral test was performed to evaluate the anxiety levels of C57BL/6X129/SV and C57BL/6 mice. Animals were placed individually for 10 min in a circular open-field arena of 40 cm in diameter surrounded by black curtains where the light intensity in the middle of the field was 30 lux. This area was divided virtually into two circular zones placed one above the other, center of 12 cm of diameter and periphery of 28 cm of diameter and once animals placed in the center, their tracking was monitored (Smart 3.0; Panlab). The time spent in these defined zones was recorded and results were measured in seconds and expressed in percentage. At least 10 animals per group were analyzed.

All spaces were properly cleaned with 96% ethanol between animals, in order to eliminate odor or other cues.

Hippocampal spine density analysis

To carry out the spine density analysis, five animals per group were used which were sacrificed by cervical dislocation. After, the brain was isolated, it was processed following the instructions of the GolgiStainTM Kit purchased from FD Neurotechnologies, Inc. (FD Rapid GolgiStainTM Kit; Cat #PK401). Images were obtained with a BX61 Laboratory Microscope (Melville NY-Olympus America Inc.). The quantification was carried out by selecting 5 neurons per animal in the dentate gyrus (DG) of the hippocampus. Measurement was done at least 50 µm from the soma along consecutive 10 µm on secondary branches starting 10 µm after branching from the primary dendrite. Spine density was calculated by dividing the number of spines per segment by the length of the segment and was expressed as the number of spines per 10 µm of dendrite.

Immunoblot blot analysis

Fresh brains of at least 4 mice per group were extracted right after euthanasia (cervical dislocation) and the hippocampus were dissected and kept frozen at -80ºC until use. Next, samples were homogenized in lysis buffer (Tris HCl 1M pH 7.4, NaCl 5M, EDTA 0.5M pH 8, Triton, distilled H20) containing protease and phosphatase inhibitor cocktails (Complete Mini, EDTA-free; Protease Inhibitor cocktail tablets, 11836170001, Roche Diagnostics GmbH, Germany ; Phosphatase Inhibitor Cocktail 3, P0044, Sigma-Aldrich, USA). The samples were centrifuged at 14,000 rpm for 10 min at 4ºC after a 30-min incubation at the same temperature. The supernatant was recovered and frozen at -80ºC until use.

Sample protein concentration was determined using the PierceTM BCA Protein Assay Kit (Thermo ScientificTM). For immunoblot assays, 10 µg per sample were used and denatured at 95ºC for 5-min in a sample buffer [0.5 M Tris HCl, pH 6.8, 10% glycerol, 2%(w/v) SDS, 5% (v/v) 2-mercaptoethanol, 0.05% bromophenol blue]. Electrophoresis was performed on acrylamide gels of 7, 10, and 12% concentration at constant 120 V and transferred to polyvinylidene difluoride sheets (Immobilon®-P Transfer Membrane; IPVH00010; Merk Millipore Ltd., USA) at constant 200 mA for 120 min. Then, membranes were blocked for 1-h with 5% non-fat milk dissolved in TBS-T buffer (0.5 mM Tris; NaCl, Tween® 20 (P1379, Sigma-Aldrich, USA), pH 7.5), washed with TBS-T 3 times for 5-min and incubated with the appropriate primary antibody, detailed in Table 1, overnight (O/N) at 4ºC. Subsequently, blots were washed in TBS-T buffer and incubated at room temperature for 1-h with the appropriate secondary antibody (Table 1). Finally, results were obtained through chemiluminescence detection using the Pierce® ECL Western Blotting Substrate (#32106, Thermo Scientific, USA), a Bio-Rad Universal Hood II Molecular Imager and the Image Lab v5.2.1 software (Bio-Rad laboratories). Measurements were expressed in arbitrary units and all results were normalized with the corresponding loading control (Glyceraldehyde-3-phosphate dehydrogenase; GAPDH).

Immunofluorescence

At least 4 animals per group were previously anesthetized by the i.p. injection of ketamine (100mg/Kg) and xylazine (10mg/Kg). When they were in the no-pain sleep phase, they were intracardially perfused with 4% paraformaldehyde (PFA) diluted in 0.1 M phosphate buffer (PB). After perfusion, brains were removed and stored in 4% PFA O/N at 4ºC. The next day, the solution was changed into 4% PFA+30% sucrose. Coronal sections of 20 µm were obtained by a cryostat (Leica Microsystems), and kept in a cryoprotectant solution at -20ºC until their use.

On the first day of the assay, free-floating sections were washed three times with 0.1 M PBS pH 7.35 and after, five times with PBS-T (0.1M PBS; 0.2% Triton X-100). Then, they were blocked in a solution containing 10% fetal bovine serum (FBS) and 1% Triton X-100 diluted with PBS-T five times for 5 min each and incubated with the primary antibody (Table 2) O/N. On the second day, slices were washed with PBS-T 5 times for 5 min and incubated with the pertinent secondary antibody (Table 2) for 2 h at room temperature. Finally, sections were treated with 0.1 µg/mL Hoechst (Sigma-Aldrich, St Louis, MO, USA) for 8 min in the dark at room temperature and washed with 0.1 M PBS. All reagents, containers and materials exposed to Hoechst were properly managed and processed to avoid any cytotoxic contamination. Finally, brain slices were mounted in gelatin-coated slides using Fluoromount G (EMS) and were left to dry O/N. Image acquisition was obtained using an epifluorescence microscope (BX61 Laboratory Microscope, Melville, NY-Olympus America Inc.) and quantified by ImageJ.

Statistical analysis

All results were represented as MEAN ± SD. Groups were compared against each other using two-way ANOVA. When variables independently were significant denote # $p<0.05$, ## $p<0.01$, ### $p<0.001$ and #### $p<0.0001$. When the overall ANOVA yielded significant effects, Tukey's post hoc test was performed for comparison between groups (* $p<0.05$. ** $p<0.01$, *** $p<0.001$ and **** $p<0.0001$). Student's t-test was used when the experiment was performed with 2 experimental groups (* $p<0.05$. ** $p<0.01$, *** $p<0.001$ and **** $p<0.0001$). All analyses and GAPDH representations were obtained using Graph Pad Prism software for Mac version 6.01; Graph Pad Software, Inc.

Results

HFD feeding induces body weight increase and glucose alterations at peripheral level

Body weight profile was analyzed at 6 months of age in WT and PPARβ/$\delta^{-/-}$ mice after being fed with conventional chow or HFD from their weaning. Two-way ANOVA revealed that feeding HFD significantly increased ($p<0.0001$) their body weight in both genotypes compared to mice fed the control diet (Fig.1a). Moreover, GTT and ITT were performed in order to evaluate peripheral alterations of glucose metabolism in these experimental groups. In line with our previous studies, two-way ANOVA showed a significant effect of diet variable in both, GTT ($p<0.0001$) and ITT ($p<0.0001$), thereby indicating that feeding the HFD affects both genotypes (Fig. 1b-e).

Evaluation of cognitive function

To determine the impact of PPARβ/δ deficiency on cognitive function together with HFD intake, we initially aimed to evaluate the hippocampal-dependent spatial learning and memory by Morris Water Maze (MWM) and learning and recognition using novel object recognition test (NORT). However, based on our experience, a behavior characterized by nervousness was detected in the C57BL/6X129/SV strain. To check this, the analysis of basal stress levels of this strain in comparison to C57BL/6 was performed by open field test. The study confirmed that C57BL/6X129/SV mice presented significant higher stress levels than C57BL/6 ($p>0.0001$) (Fig.2). For this reason, we considered that behavioral tests were not suitable to evaluate cognition in this strain since stress interferes in the obtained results.

PPARβ/δ$^{-/-}$ mice and obesity are associated with decreased dendritic spine density

Due to the limitations observed in relation with the behavioral tests, the cognitive process was evaluated by the analysis of dendritic spine density in the hippocampus, since it has been demonstrated that the number of dendritic spines positively correlates with synaptic plasticity and cognition (21,22). In our study, the dendritic spine detection and subsequent quantification was performed where the results obtained demonstrated a significant effect of diet and genotype ($p<0.01$ and $p<0.0001$, respectively) with the interaction between both variables ($p<0.01$). Following, Tukey´s post-hoc was performed and our results showed a significant decrease of hippocampal spine number in WT mice after being fed with HFD ($p<0.0001$). By contrast, animals with PPARβ/δ deficiency also exhibited a significant reduction compared to WT, but HFD feeding did not induce a synergic effect (WT CT *vs.* PPAR β/δ$^{-/-}$ CT $p<0.001$, WT CT *vs.* PPAR β/δ$^{-/-}$ HFD $p<0.0001$) (Fig.3a-b). Moreover, as can be observed in Fig. 3a, the reduction of spine number was accompanied by shorter and smaller dendritic spines (qualitative evaluation). Therefore, our results indicated that HFD feeding induces cognitive alterations in WT. However, when animals exhibit PPARβ/δ deficiency, they showed cognitive alterations which were not enhanced by the diet.

In order to support these findings, proteins directly involved in cognitive process and plasticity in the hippocampus such as the drebrin 1 (DBN1), neurexin, density protein 95 (PSD95), synaptophysin and brain-derived neurotrophic factor (BDNF) were evaluated. DBN1 has been demonstrated to play a key role in dendritic spine regulation (23,24). In this line, two-way ANOVA revealed a significant reduction ($p<0.01$) in DBN1 protein levels in PPARβ/δ$^{-/-}$ mice compared with WT mice. Likewise, neurexin (a presynaptic protein) showed the same pattern, a significant implication of the genotype ($p<0.01$) in its protein level. Moreover, a significant effect of the genotype ($p<0.05$) also was observed in PSD95, (a postsynaptic protein) but in this case with interaction between two variables ($p<0.05$). Therefore, Tukey's post-hoc was carried out to analyze the differences among the experimental groups. Our results exhibited a significant reduction in PSD95 in PPARβ/δ$^{-/-}$ CT compared to WT CT. Regarding synaptophysin, this protein is expressed in synaptic vesicles and its reduction has been associated to impairments to neuronal health together with BDNF (25). In this line, our results showed a significant reduction of synaptophysin in those animals fed with HFD, demonstrating a significant effect of this variable ($p<0.05$), but this reduction was not exacerbated by the absence of PPAR β/δ. Finally, concerning BDNF, results showed a significant

effect of the diet ($p<0.001$) and genotype ($p<0.01$) with the interactions between both variables ($p<0.001$). Next, Tukey´s post-hoc test revealed a significant decrease in hippocampal BDNF protein levels in PPARβ/$\delta^{-/-}$ mice fed with HFD compared to the other groups (WT CT vs PPAR β/$\delta^{-/-}$ HFD $p<0.001$; WT HFD vs PPAR β/$\delta^{-/-}$ HFD $p<0.001$; PPAR β/$\delta^{-/-}$ CT vs PPAR β/$\delta^{-/-}$ HFD $p<0.0001$) (Fig. 4). Collectively, as previously described (26), our results confirm that HFD feeding contributes to disturbances in the synaptic transmission. However, when there is a PPAR β/δ deficiency, biomarkers related to neuronal function are directly altered independently of diet, suggesting the essential role that PPAR β/δ plays in the synaptic transmission.

PPAR β/δ deficiency increases glial markers activation

Evaluation of the reactive profile of astrocytes and microglia in the hippocampal dentate gyrus was performed through the detection of glial fibrillary acidic protein (GFAP) and ionized calcium-binding adapter molecule 1 (IBA1) proteins by immunohistofluorescence. Representative images of all four experimental groups can be found in Fig. 5. Moreover, graphic quantification of fluorescence intensity measured by ImageJ is shown in Fig. 5i for GFAP and j for IBA1. In the case of GFAP, two-way ANOVA showed a significant effect of diet ($p>0.05$) and genotype variables ($p<0.01$) without interaction between them, demonstrating that, on the one hand, HFD induced a significant activation of astrocytes compared to control diet, and on the other, PPARβ/$\delta^{-/-}$ mice presented a significantly higher basal profile of reactive astrocytes than WT mice (Fig 5i). In the study of microgliosis, our results revealed a significant effect of both, diet ($p<0.01$) and genotype ($p<0.0001$) with an interaction between both factors ($p<0.0001$). Subsequently, Tukey´s post-hoc showed a significant microgliosis in WT mice fed with HFD compared to those fed with conventional chow ($p<0.0001$). In the case of PPAR β/$\delta^{-/-}$ mice, both groups exhibit a significant microglial activation compared to WT regardless of the consumed diet (PPARβ/$\delta^{-/-}$ CT vs WT CT $p<0.0001$; PPARβ/$\delta^{-/-}$ HFD vs WT CT) (Fig.5j).

At the molecular level, the levels of different proteins related to the neuroinflammation process were analyzed in the hippocampus including toll-like receptor 4 (TLR4), nuclear factor kappa B(NFKb) and protein tyrosine phosphatase (PTP1B). Regarding TLR4 protein levels, two-way ANOVA showed a significant effect of genotype ($p<0.05$) with the interaction between both variables, genotype and diet ($p<0.05$). Next, Tukey´s post-hoc revealed a significant TLR4 increase in PPARβ/$\delta^{-/-}$ mice compared to WT fed the standard chow diet (WT CT vs PPARβ/$\delta^{-/-}$ CT $p<0.001$; WT CT vs PPARβ/$\delta^{-/-}$ HFD $p<0.01$) (Fig.6).

In the case of NFKb and PTP1B, two-way ANOVA revealed a significant effect of diet and genotype for both proteins ($p<0.05$ and $p<0.05$; $p<0.01$ and $p<0.05$, respectively), although none of them demonstrated interaction between both factors (Fig.6). Collectively, our results confirm that feeding the HFD and the lack of PPARβ/δ differently affect the protein levels of proteins involved in neuroinflammation.

PPAR β/δ deficiency disrupts the insulin signaling pathway in the hippocampus

Since previous studies have demonstrated that hippocampal insulin signaling plays a key role in the cognitive processes (27–29), we next evaluated different proteins involved in this molecular pathway, including glycogen synthase kinase 3 beta (GSK3b) and protein kinase B (AKT). The phosphorylation levels of these proteins showed a similar profile. When two-way ANOVA was applied, a significant effect of the genotype was observed ($p<0.01$ and $p<0.01$) without interaction between genotype and diet, thereby suggesting that PPARβ/δ deficiency reduces their activation. However, GSK3b and AKT did not show a significant effect on variables after statistical analysis. Surprisingly, when insulin receptor (IR) was evaluated, results showed that the genotype significantly affected IR levels ($p<0.05$), probably to compensate for the inefficacy of the pathway (Fig. 7).

Discussion

The present work provides novel findings on the neurological role of PPARβ/δ in mice under standard conventional chow diet and after HFD intake, which indicate that PPARβ/δ plays a prominent role in dendritic spine preservation and, therefore, may protect against the cognitive decline process. Interestingly, HFD consumption does not exacerbate brain cognitive pathology observed in the PPARβ/δ-deficient mice. Thus, using the well-established line of PPARβ/δ $^{-/-}$ mice and its WT controls, we assessed the impact of PPARβ/δ deficiency on cognitive function, synaptic plasticity, dendritic spine density, and synaptic markers and evaluated whether HFD intake deteriorates this pathological status.

It has been widely demonstrated that the PPAR superfamily plays a key role in metabolic processes. In fact, their agonists are used for the treatment of pathologies including T2DM. Specifically, it has been demonstrated that PPARβ/δ is expressed throughout the brain, with prominent localization in the mouse hippocampus, entorhinal cortex, and hypothalamus (30–32). Interestingly, several studies have demonstrated the link between metabolic dysfunctions and cognitive decline. In fact, AD is considered as type 3 diabetes (33, 34). Therefore, taking all these into account, the interest in this receptor and its agonists has increased for potential therapeutic interventions in the treatment of AD and cognitive disorders related to obesity.

PPAR β/δ agonists substantially decrease adiposity and improve glucose intolerance and insulin resistance in animal models (4, 17, 35–37). In line with previous studies (38, 39), feeding the HFD caused a significant increase in body weight, glucose intolerance and insulin resistance. However, we did not find alterations in these parameters due to the genotype. One possible explanation for these discrepancies could be the differences in time exposition to the HFD. In this context, previous studies have exposed animals to a hypercaloric diet following a different pattern compared to ours in which animals were fed from their weaning at 21 days-old until their sacrifice at 6 months. Therefore, in line with previous studies, young animals exposed to a HFD might show more plasticity to adapt to the diet (40).

It has been widely demonstrated that PPARβ/δ plays a key role in running endurance (41–45). Therefore, the lack of PPARβ/δ may affect the swimming performance making MWM unreliable to assess the mice cognition. Moreover, since it has been described that behavior alterations could be observed among

hibrids/strains (46), we used the open field test to assess whether C57BL/6X129/SV mice showed anxiety levels that may also affect the MWM or another behavioral test to evaluate cognition in this strain. Our results demonstrated that C57BL/6X129/SV showed significant increase levels of anxiety compared to C57BL/6. These data reinforce the stance to evaluate cognitive process using alternative techniques such as dendritic spine evaluation.

It is well known that dendritic spines play a pivotal role in the learning process, whereas synaptic plasticity alterations have been directly correlated to memory impairments (47–52). This prompted us to focus our study on the evaluation of these structures in order to assess the effect of PPARβ/δ deficiency in dendritic spines density at the hippocampal level. In this line, our results highlighted that PPARβ/$\delta^{-/-}$ mice showed a significant reduction of these structures in mice fed a standard diet and this was not exacerbated by the HFD, suggesting that this receptor is necessary for the maintenance of dendritic spines and, in consequence, for eluding cognitive decline. On the other hand, it is known that obesity-associated with HFD intake heightens neuronal loss and cognitive decline through several mechanisms, including neuroinflammation (53, 54). Our results confirm this neuronal loss in WT fed with HFD compared to WT mice fed with the standard chow.

Going in-depth at the molecular level, DBN1 is an actin-binding protein abundant within dendritic spines, which is typically located in postsynaptic receptive regions of excitatory synapses (55) and it is thought that it controls spine morphology and function (56). In fact, its reduction in the hippocampus has been correlated with cognitive deficits and, by contrast, its preservation has been associated to neuroprotection (57–60). In line with these previous studies, our study showed a significant decrease in DBN1 protein level in the hippocampus induced by PPARβ/δ deficiency. This is in agreement with the findings obtained in dendritic spine analysis. Likewise, it has been reported that *dbn1* loss in the brain is not sufficient to induce synaptic dysfunction (61). Therefore, our study suggests that PPARβ/δ actively contributes to the preservation of these structures together with DBN1. Consistent with this, neurexin and PSD95 protein levels also showed a similar pattern, which confirms that PPARβ/δ plays a pivotal role in synaptic plasticity.

Regarding synaptophysin, the reduction of this glycoprotein, which predominates in the synaptic vesicles, has been related not only to cognitive decline but also to the development of anxiety (62, 63). In this context, obesity has also been associated with anxiety behavior interconnecting these 3 concepts (64, 65). Our results showed a significant decrease in synaptophysin protein levels in the hippocampus induced by the HFD feeding. However, we did not observe any significant reduction related to the genotype, probably due to the increased anxiety observed in C57BL/6X129/SV strain. The neuronal activity has also been described to regulate BDNF transport into dendrites, which have been involved in the modulation of synaptic transmission and synaptogenesis (66–68). In this study, we observed a significant reduction of its levels in PPARβ/$\delta^{-/-}$ fed with HFD compared to the other groups, suggesting that PPARβ/δ deficiency alone is not enough to alter BDNF protein levels at this time.

Neuroinflammation is a common feature of every central nervous system diseases and is being highly recognized as a potential mediator of cognitive impairment (69, 70). The impact of this complex process, which includes the alteration of the TLR4 pathway and glial activation among other processes, induce the release of pro-inflammatory cytokines and aberrant neuronal circuits, contributing altogether to the acceleration of cognitive decline (71–73). However, the involvement of inflammation is not fenced only in the brain. In fact, it has been demonstrated that HFD intake and obesity also impair cognitive function in animal models (74), as well as in humans (75), by disrupting hippocampal morphology and synaptic plasticity caused by inflammation (76, 77). In this context, PPARβ/δ has been involved in the modulation of inflammation at both, peripheral and central levels. Our study demonstrated that the lack of PPARβ/δ receptor enhances the gliosis in the hippocampus, contributing to astrocyte and microglial activation. Likewise, TLR4 and NFKβ protein levels also showed a significant increase in PPAR $\beta/\delta^{-/-}$ mice. In agreement with these data, studies performed by Rodríguez-Calvo and colleagues demonstrated that the PPARβ/δ agonist GW501516 inhibited LPS-induced cytokine production by preventing NFKβ activation (78). Interestingly, HFD promoted similar alterations to those observed when there is a deficiency of PPARβ/δ. Exceptionally, in the case of microglial activation, whereas feeding HFD induced neuroinflammation in WT mice, feeding KO mice with HFD did not exacerbate these changes, similar to TLR4.

Interestingly, our results showed a significant increase of PTP1B protein levels in the hippocampus caused by the genotype. This increase was similar to that observed after HFD intake. An analogous trend was observed for NFKβ and astrogliosis. PTP1B expression is highly increased in activated microglia, which in turn is enhanced due to pro-inflammatory processes, suggesting that is an important positive regulator of inflammation (79). However, PTP1B has not only been related to neuroinflammatory mechanisms. In fact, it has been demonstrated that it is a key regulator of insulin sensitivity since mice with *Ptp1b* gene deletion present a reduction of insulin resistance, turning it into a promising target not only for the design of anti-diabetic drugs (80, 81), but also to elude synaptic alteration and cognitive loss (82, 83), since PTP1B activity in the hippocampus has been correlated with impaired neuronal insulin signaling (63). Taking all these data into account, our results confirm the studies performed by de la Monte and colleagues that demonstrated that downregulation of PPARβ/δ could be linked to both, inflammation and insulin resistance in the brain (13).

It is well known that IR and insulin signaling play an important role in neuronal physiological functions, contributing to synapse formation, neuronal plasticity (84, 85) and reduction of neuroinflammatory process, which all together promote the cognitive function (86–88). Once IR is activated, AKT is phosphorylated/activated, which in turn phosphorylates various biological substrates, including GSK3β. In this context, the dysfunction of this pathway occurs, which has been associated with insulin resistance leading to cognitive decline (89–91). In our study, p-AKT and p-GSK3β protein levels showed a significant decrease in the hippocampus of PPARβ/$\delta^{-/-}$ mice in agreement with spine dendritic reduction and increased neuroinflammation observed in this genotype. Moreover, we did not observe changes in the diet variable, probably due to the fact that the lack of this receptor interferes in the attachment of fat to its

receptor. Of note, an increase in IR protein level was observed in these mice, suggesting that it could be a compensatory mechanism to deal with this insulin signaling disruption. These results concur with previous studies performed by Buck and colleagues who demonstrated that when insulin-like growth factor 1 receptor (a co-receptor of insulin signaling pathway) was inhibited, a compensatory IR activation was observed (92), demonstrating the key role of PPARβ/δ in this process.

Conclusions

In conclusion, the present study demonstrates for the first time that PPARβ/δ deficiency in the brain constitutes not only a new risk factor associated with cognitive loss in neurological diseases, but also a key molecule targeting the pivotal pathways leading to cognitive decline which include neuroinflammation, insulin resistance, dendritic spine regulation and synaptic plasticity, among others. Therefore, PPARβ/δ provides a new and promising therapeutic target in order to design novel strategies focused on curbing or improving cognitive decline present in most neurological diseases.

Abbreviations

AD	Alzheimer´s disease
AKT/p-AKT	Protein kinase B/ phospho- Protein kinase B
BDNF	Brain-derived neurotrophic factor
CNS	Central nervous system
CT	Conventional chow
DBN1	Drebrin 1
DG	Dentate gyrus
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GFAP	Glial fibrillary acidic protein
GSK3β/p-GSK3β	Glycogen synthase kinase 3 beta
GTT	Glucose tolerance test
HFD	High fat diet
IBA1	Ionized calcium-binding adapter molecule 1
IR	Insulin receptor
ITT	Insulin tolerance test
KO	Knock-out
MWM	Morris Water Maze
NFKβ	Nuclear factor kappa B
NORT	Novel object recognition test
NSAIDs	Nonsteroidal anti-inflammatory drugs
PB	Phosphate buffer
PFA	Paraformaldehyde
PPARs	Peroxisome proliferator-activated receptors
PSD95	Density protein 95
PTP1B	Protein tyrosine phosphatase
T2DM	Type 2 Diabetes Mellitus
TLR4	Toll-like receptor 4
WT	Wild type

Declarations

Ethics approval and consent to participate

All the experiment on APP/PS1 transgenic mice were performed in accordance with the European Community Council Directive 86/609/EEC and the procedures were established by the Department d'Agricultura, Ramaderia i Pesca of the Generalitat de Catalunya.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article

Competing interests

The authors declare that they have no conflict of interest.

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Authors' contributions

TE-J performed experiments, collected and analyzed the data, editing figures, bibliography search, writing-original draft section. OB performed experiments, bibliography search, writing-original draft section. AC manuscript revision, experiment supervision, bibliography search. EV manuscript revision, experiment supervision. JO manuscript revision, linguistic correction. CA manuscript revision, experiment supervision. JF manuscript revision. WW manuscript revision, linguistic correction, bibliography search. MV-C manuscript revision, linguistic correction, bibliography search. AC proposal and schematization of the article, funding acquisition, manuscript revision, bibliography search. ME schematization of the article, writing-original draft, bibliography search. All authors read and approved the final manuscript.

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Not applicable

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Tables

Due to technical limitations, tables are only available as a download in the Supplemental Files section.

Figures

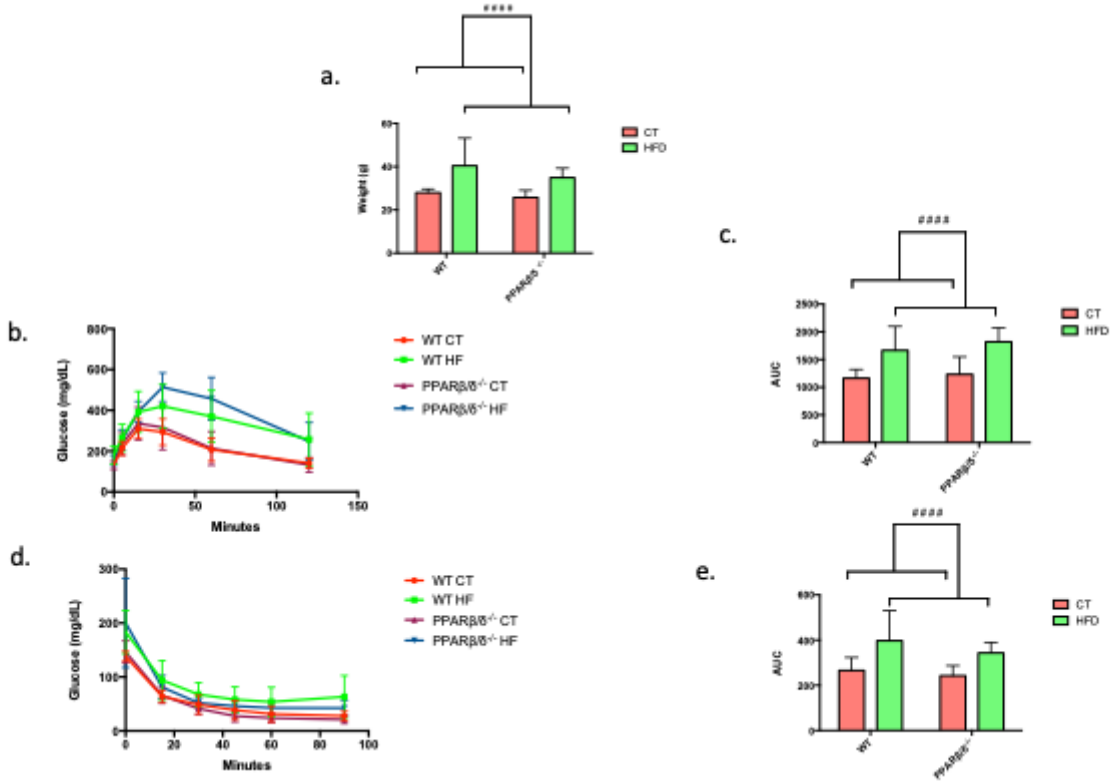

Figure 1

a. Analysis and representation of changes in body weight (n=12 animals per group). b. GTT and d. ITT experiment profiles (n=12 animals per group). AUC data was calculated from the time point 0 until the end of the experiment for both c. GTT and e. ITT. Statistical analysis was performed through two-way ANOVA. Significant differences were found between control and high fat diet groups: # # # # denote p< 0.0001.

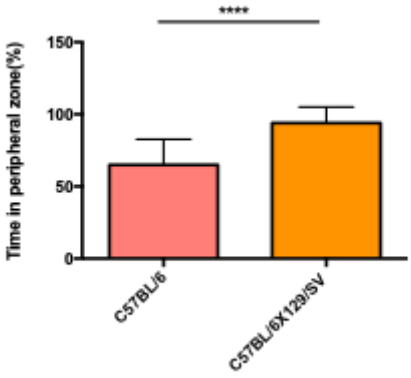

Figure 2

Graphic representation of anxiety levels (Number of animals ≥ 10). Statistical analysis was performed by Student's t test. Significant differences were found between strains: **** denote p<0.0001.

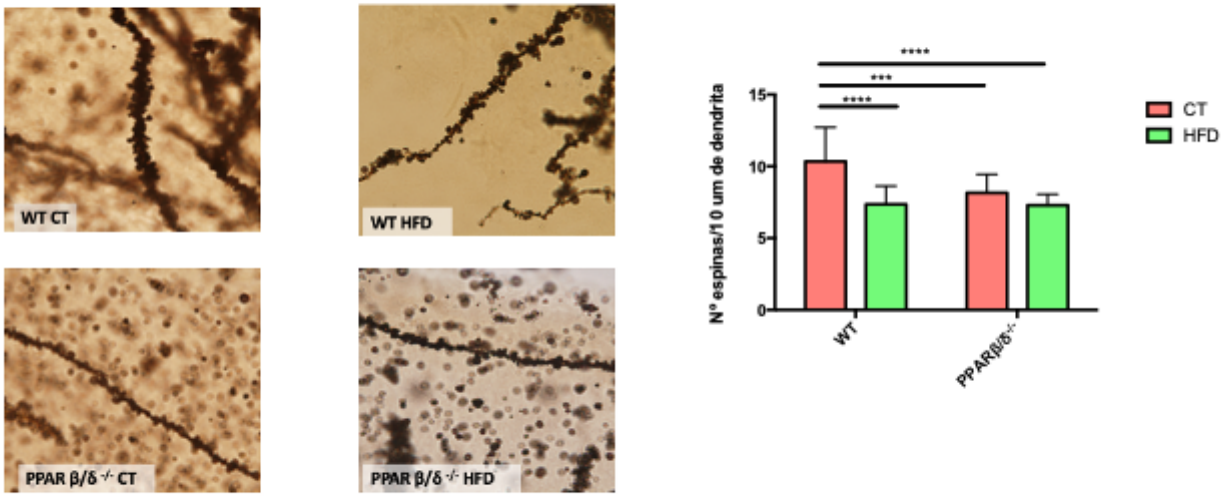

Figure 3

(a) Optical microscope images of brain GolgiStain. Scale bar: 10 μm. (b) Quantification of dendritic spines for each 10 μm. Groups were compared against each other using two-way ANOVA and Tukey post-test (n=5) (WT CT vs WT HFD: **** denote p<0.0001; WT CT vs PPAR β/δ-/- CT: *** denote p<0.001; WT CT vs PPAR β/δ-/- HFD: **** denote p<0.0001).

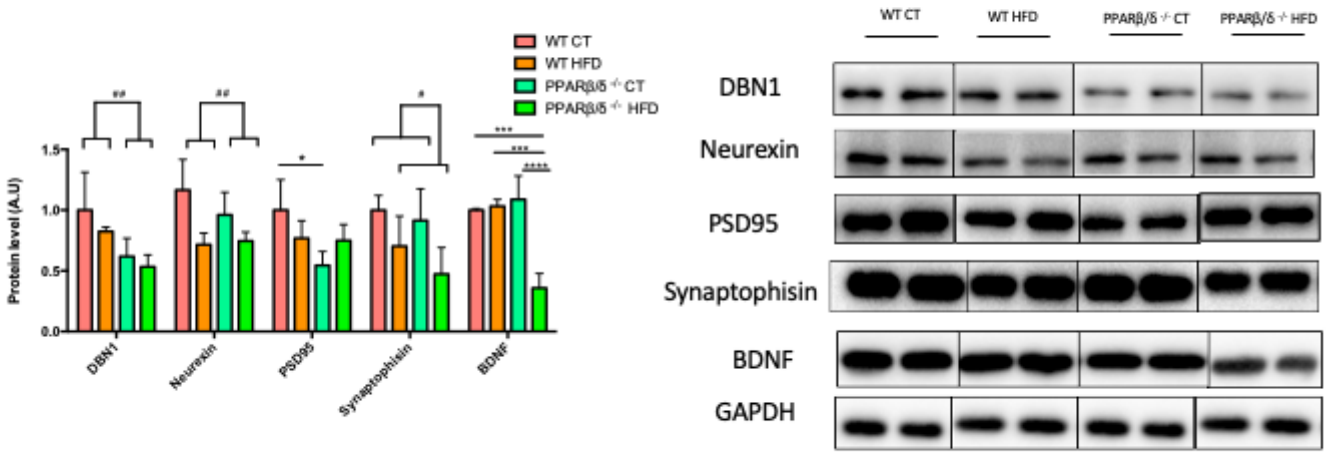

Figure 4

Immunoblot detection of synaptic proteins where two representative samples out of four per group are shown. All results were represented as MEAN ± SD. Groups were compared against each other using two-way ANOVA (n≥4) (CT vs HFD: # denote p<0.05) (WT vs PPAR β/δ-/-: ## denote p<0.01). In the case of PSD95 and BDNF, Tukey post-test was performed (PSD95: WT CT vs PPAR β/δ-/- CT: *denote p<0.05) (BDNF: WT CT vs PPAR β/δ-/- HFD: *** denote p<0.001; WT HFD vs PPAR β/δ-/- HFD: *** denote p<0.001; PPAR β/δ-/- CT vs PPAR β/δ-/- HFD: **** denote p<0.0001

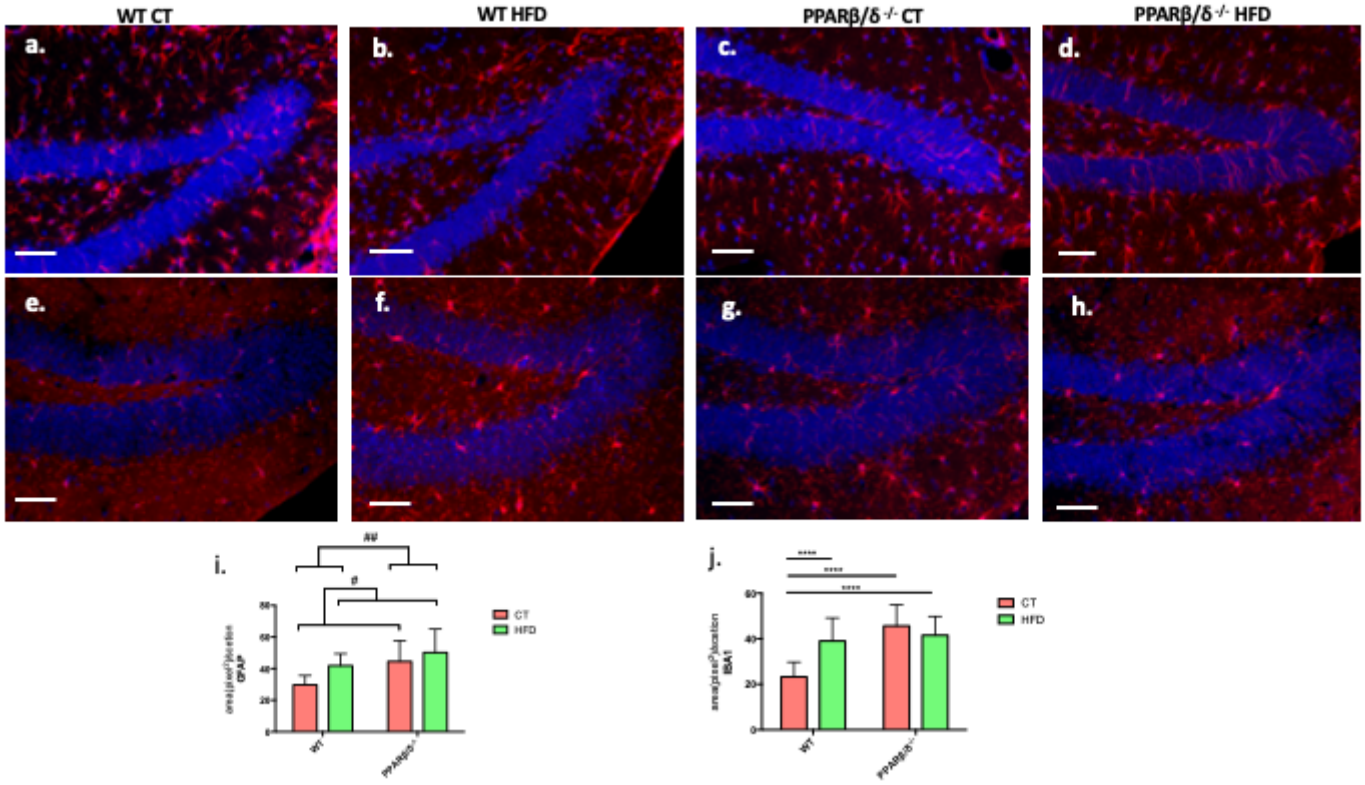

Figure 5

Evaluation of inflammatory responses. Representative images for the detection of astrocytes (a-d) and microglia (e-h) (red color in both cases) in the DG of the hippocampus. All samples are co-stained with Hoechst for the detection of cellular nucleus (blue). Scale bar: 200 μm. Graphic representation of fluorescence intensity for GFAP (i) and IBA1 (j). In the case of GFAP, statistical analysis was performed through two-way ANOVA (Number of animals ≥4) (Control diet vs HFD: # denote p<0.05) (WT vs PPAR β/δ-/- : ## denote p<0.01). For IBA1, two-way ANOVA and Tukey's were used for statistical analysis (WT CT vs WT HFD: **** denote p<0.0001, WT CT vs PPAR β/δ-/- CT: **** denote p<0.0001, WT CT vs PPAR β/δ-/- HFD: **** denote p<0.0001).

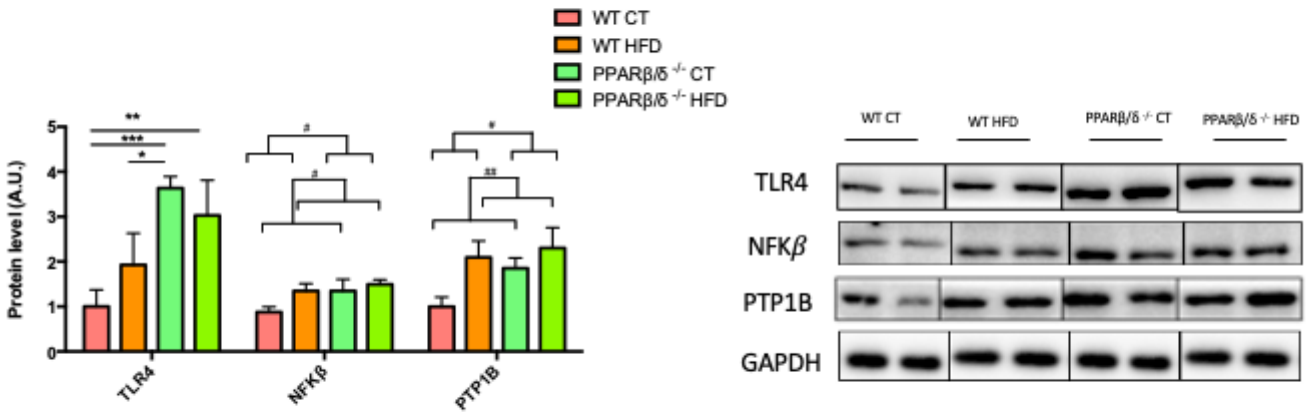

Figure 6

Semi-quantification of protein levels for TLR4, NFKβ and PTP1B where two representative samples out of four per group are shown. All results were represented as MEAN ± SD. Groups were compared against each other using two-way ANOVA (n≥4) (CT vs HFD: ## denote p<0.01; WT vs PPAR β/δ-/- : # denote p<0.05). In the case of TLR4, Tukey post-test was performed (WT CT vs PPAR β/δ-/- HFD: ** denote p<0.01; WT CT vs PPAR β/δ-/- CT: *** denote p<0.001; WT HFD vs PPAR β/δ-/- CT:* denote p<0.05).

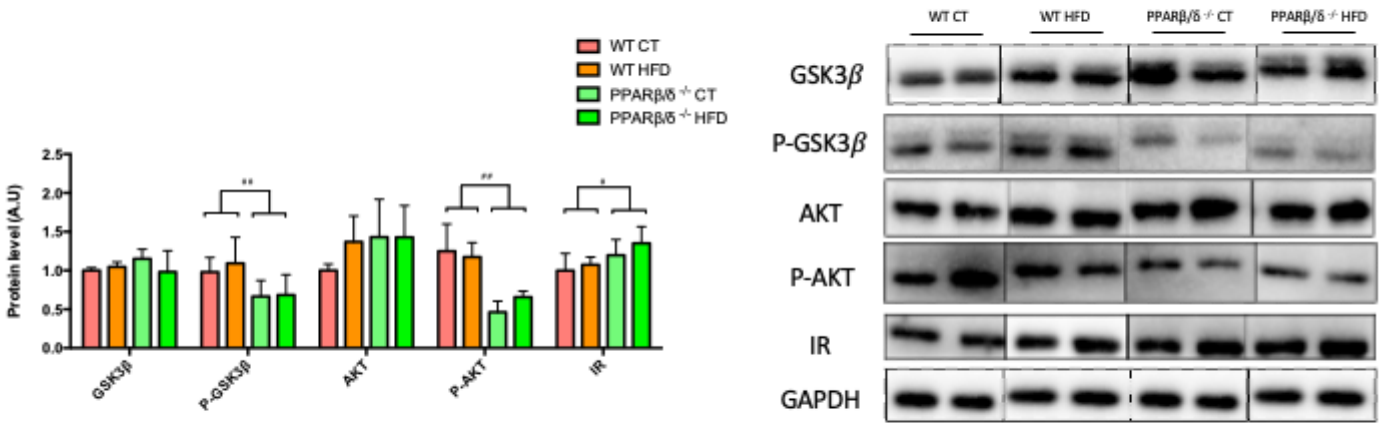

Figure 7

Immunoblot detection of IR and related signaling proteins where two representative samples out of four per group are shown. All results were represented as MEAN ± SD. Groups were compared against each other using two-way ANOVA (n ≥4) (WT vs PPAR β/δ-/-: # #denote p<0.01; # denote p<0.05).

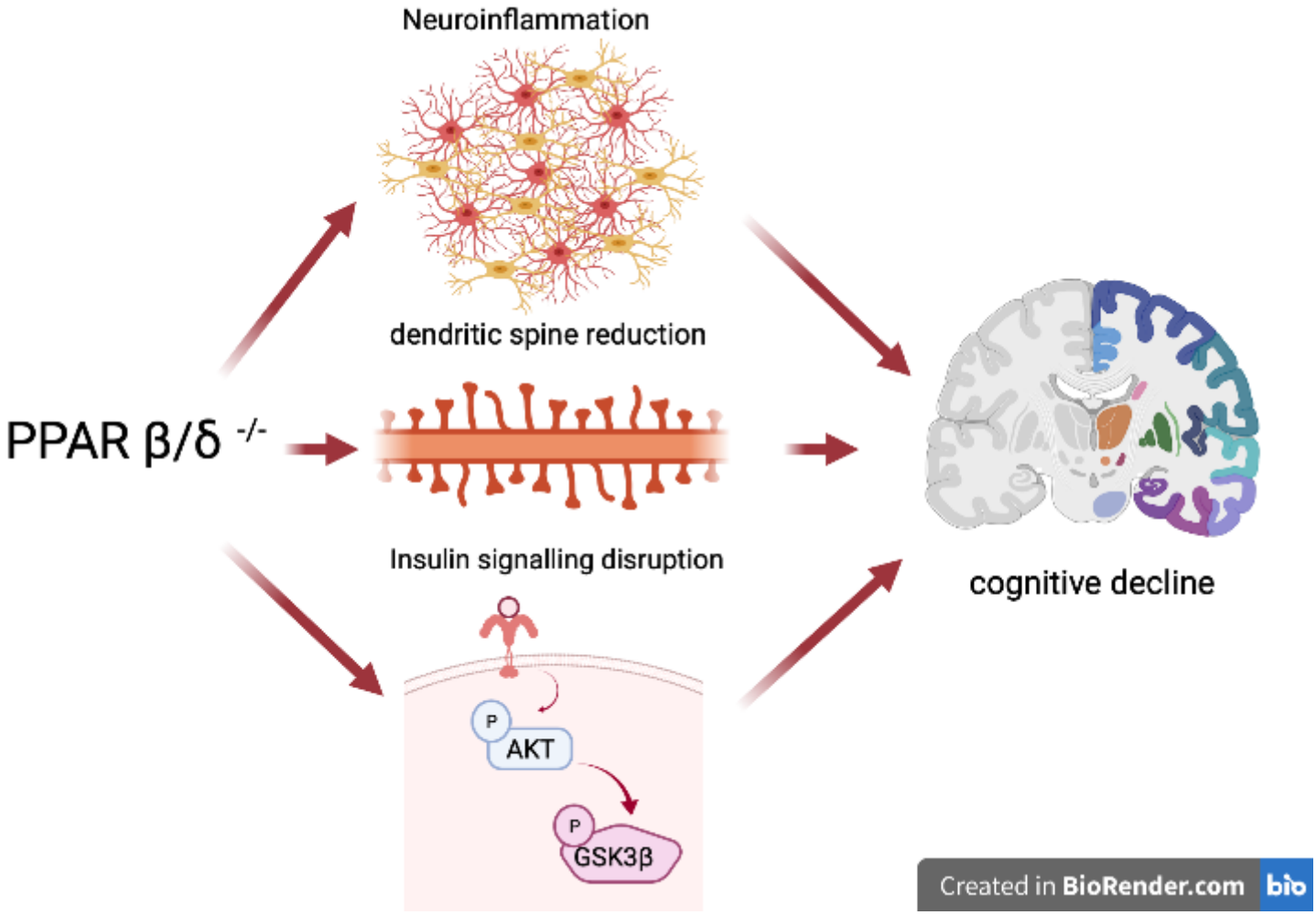

Figure 8

Schematic representation of the effects of PPARβ/δ deficiency. The figure shows how this receptor is a key molecule in the development of some of the most important features of cognitive decline such as neuroinflammation, reduction in the number of dendritic spines, as well as, an alteration of synaptic biomarkers, and insulin signaling disruption. In this way, this transcription factor represents a promising target for the treatment and improvement of cognitive decline, an important hallmark of neurodegenerative diseases.

Supplementary Files

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- Table1.tiff
- Table2.tiff