PPAR γ Dysfunction in the Medial Prefrontal Cortex Mediates High-Fat Diet-Induced Depression

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Research Article

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Abstract

Objective

Epidemiological studies suggest a bidirectional association between depression and obesity; however, the biological mechanisms that link the development of depression to a metabolic disorder remain unclear. Even though nuclear receptor peroxisome proliferator-activated receptor γ (PPAR γ) agonists show anti-depressive effect, and high-fat diet (HFD)-induced PPAR γ dysfunction is involved in the pathogenesis of metabolic disorders, the neuronal PPAR γ has never been studied in HFD-induced depression. Thus, we aimed to investigate the effect of neuronal PPAR γ on depressive-like behaviors in HFD-induced obese mice.

Methods

We fed male C57BL/6J mice with HFD to generate obese mice and conducted a series of behavioral tests to assess the effects of HFD feeding on depression. We generated neuron-specific PPAR γ knockout mice (NKO) to determine whether neuronal PPAR γ deficiency was correlated with depressive-like behaviors. To further prove whether PPAR γ in the medial prefrontal cortex (mPFC) neurons is involved in depressive-like behaviors, we applied AAV- CaMKIIa-Cre approach to specifically knockout PPAR γ in the mPFC neurons of LoxP mice and used AAV-syn-PPAR γ vectors to overexpress PPAR γ in the mPFC neurons of NKO mice.

Results

We observed a low mPFC PPAR γ level and an increase in depressive-like behaviors in the HFD-fed mice. Moreover, neuronal-specific PPAR γ deficiency in mice induced depressive-like behaviors, which could be abolished by imipramine. Furthermore, overexpressing PPAR γ in the mPFC reversed the depressive-like behaviors in HFD-fed mice as well as in neuronal-specific PPAR γ knockout mice.

Conclusions

These results implicate that dysregulation of neuronal PPAR γ in the mPFC may contribute to an increased risk for depression in obese populations.

Introduction

Depression is a chronic and debilitating mental illness with a 17% lifetime prevalence and is a major cause of morbidity, disability and mortality [1]. Clinical studies have demonstrated that depressed patients exhibit multiple symptoms, and a significant percentage of depressed patients are refractory to classical monoamine-based antidepressants, suggesting that the pathophysiology leading to depression may differ among patients [2]. Increasing evidence has suggested that depression may involve different molecular mechanisms [3]. Identifying the pathophysiology and signaling pathways underlying the multiple causes of depression is necessary to effectively control depression.

Previous work has suggested a bidirectional association between depression and obesity. Obese individuals have a 55% increased risk of developing depression over time [4], and diabetes is estimated to double the incidence of depression [5,6]. At the same time, depressed individuals have a 58% increased risk of becoming obese. Depression is considered a systemic disease, and metabolic diseases, such as obesity, insulin and leptin resistance, may also be responsible for the increased incidence of depression [7,8]. Therefore, elucidating the biological mechanisms that promote depressed moods in individuals with obesity/diabetes is useful to find targets for new agents.

The nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR γ) is a ligand-activated transcription factor that belongs to the nuclear receptor family, and the activity of which is regulated by the direct binding of steroid and thyroid hormones, vitamins, lipid metabolites, and xenobiotics [9]. Impaired PPAR γ activity has been observed in the adipocytes of obese mice, and this impairment mediates insulin resistance [10]. Moreover, the Pro12Ala polymorphism in PPAR γ is associated with a high risk for developing depression [11]. Furthermore, Ahmed et al. reported that rosiglitazone produces a specific antidepressant-like effect in the forced swim test (FST) and tail suspension test (TST) [12]. Guo et al. demonstrated a novel role for adipose PPAR γ in susceptibility and resilience to chronic stress and a functional link between PPAR γ and adiponectin in mediating depressive-like behaviors [13]. Neuronal seipin deficiency, which causes reduced PPAR γ levels, leads to depressive-like behaviors in male mice that are reversed by PPAR γ agonist rosiglitazone [14]. However, whether neuronal PPAR γ impairment is sufficient to induce depressive like behaviors, or whether neuronal PPAR γ deficiency is involved in the etiology of depression, have not been proven.

In the current study, we examined whether high-fat diet-(HFD)-induced obesity causes a decrease in brain PPAR γ and whether this impairment of PPAR γ activity contributes to the onset of depression. We found that HFD-induced obese mice displayed lower PPAR γ levels in their medial prefrontal cortex (mPFC) and depressive-like symptoms, which could be blocked by AAV-PPAR γ stereotactically injected into the mPFC. Furthermore, we demonstrated that mPFC neuronal-specific PPAR γ -deficiency is sufficient to induce depressive-like behaviors, which could be rescued by overexpressing PPAR γ .

Materials And Methods

Animals. Adult male C57BL/6J mice (aged 7-8 weeks) were obtained from the Southern Medical University Animal Center (Guangzhou, China). Retired male CD1 (aged 8-9 months) breeders were obtained from Charles River Laboratories (Beijing, China). PPAR γ LoxP/LoxP (PPAR $\gamma^{\text{fl/fl}}$) mice and calcium/calmodulin dependent protein kinase II alpha (CaMKIIa)-Cre mice [15] were purchased from the Jackson Laboratory (Maine, USA). Neuronal-specific PPAR γ knockout mice (NKO) [16] were generated by crossing the PPAR $\gamma^{\text{fl/fl}}$ mice with the CaMKIIa-Cre mice. PPAR $\gamma^{\text{fl/fl}}$ (LoxP) mice were used as the control. Mice were housed four per cage in standard laboratory cages except for CD1 breeders, which were individually housed in the same kind of cages. All mice were kept on a standard 12 h light/dark cycle (8:00-20:00 light period) in a temperature-controlled room ($21 \pm 25^\circ\text{C}$), with free access to food and water. All of the experiments were conducted in accordance with the regulations of the Administration of

Affairs Concerning Experimental Animals (China) and were approved by the Southern Medical University Animal Ethics Committee. Male mice were used in all experiments.

Genotype identification of PPAR γ gene knockout mice. The offspring of the transgenic mice were genotyped using PCR with mouse tail DNA and the DNA was amplified with the following primers: 5'-TGCCAAGAAGAAGAGGAA-3' (forward primer for CaMKIIa-Cre) and 5'-TTGCAGGTACAGGAGGTAGTC-3' (reverse primer for CaMKIIa-Cre); 5'-TGTAATGGAAGGGCAAAGG-3' (forward primer for LoxP) and 5'-TGGCTTCCAGTGCATAAGTT-3' (reverse primer for LoxP). The PCR products were visualized using ethidium bromide staining.

Obesity model. One week after acclimating to current condition, the male C57BL/6J mice were randomly divided into 2 groups and fed either with control diet (CD) (10% kcal fat, D12450B, Research Diets) or HFD (60% kcal fat, D12492, Research Diets) for 16 consecutive weeks. After the HFD feeding regimen, all mice were evaluated with glucose tolerance test, behavioral tests or virus administration.

Glucose tolerance test. Mice were fasted overnight (15 h) before undergoing glucose tolerance test (GTT). Glucose (2 g/kg; Sigma-Aldrich) was injected intraperitoneally, and blood samples were obtained before (0) and 30, 60, 90 and 120 min after the injection of glucose. Blood glucose was measured using a glucometer (Bayer HealthCare LLC, 1455).

Primary cortical neuron cultures. The neuronal culture was prepared from the cortices of littermates (within 24 h after birth) of NKO or PPAR $\gamma^{fl/fl}$ mice as described with modifications [17]. Briefly, dissociated cortical neurons were cultured in Neurobasal medium supplemented with 2% B27 and 25 μ M glutamate in poly-L-lysine-coated plates. After 24 h in culture, the cells were treated for 48 h with 0.1 μ M cytosine arabinoside (Ara-C) to inhibit glial growth. PPAR γ expression in the neuronal cultures was determined 15 days after culture *in vitro*.

Stereotaxic surgery.

Virus generation and stereotaxic injections: For PPAR γ overexpression, pAAV-syn-EGFP-T2A-PPAR γ -WPRE-pAS (AAV-PPAR γ) and pAAV-syn-EGFP-T2A-WPRE-pAS (AAV-EGFP) viruses were packaged by Shi-Ao Bioscience (Shanghai, China). For knockdown of PPAR γ , PAOV-CaMKIIa-EGFP-T2A-Cre (CaMKIIa-Cre) and PAOV-CaMKIIa-EGFP-T2A-3FLAG (CaMKIIa-EGFP) viruses were purchased from Obio Technology (Shanghai, China). The titers (genome copies per milliliter) of the AAVs were as follows: 1.51×10^{13} for PAOV-CaMKIIa-EGFP-T2A-Cre (CaMKIIa-EGFP), 1.5×10^{13} for PAOV-CaMKIIa-EGFP-T2A-3FLAG (CaMKIIa-EGFP). A total volume of 0.5 μ l of PPAR γ overexpression AAV vectors or 1.0 μ l of PPAR γ knockdown Cre viruses was delivered into the mPFC region (AP=+1.78, ML=0.30, DV=2.65) at 0.1 μ l/min with a Hamilton syringe fitted with a 33-gauge needle that was filled with 5.0 μ l of virus. The injection needle was withdrawn 10 min after the infusion. Behavioral experiments were conducted 3 weeks after the viral injection. Then, C57BL/6J mice were perfused transcardially, and serial brain sections were generated. Fluorescence microscopy and Western blotting were performed to observe the effects of transfection *in vivo*.

Tissue preparation and immunofluorescence. The brain tissue was prepared as described previously [18]. Adult C57BL/6J mice injected with AAV were anaesthetized with isoflurane and sacrificed by intracardial perfusion with heparinized 0.9% saline following by ice-cold 4% paraformaldehyde. The brains were removed and post-fixed with 4% paraformaldehyde. The fixed brains were washed with flowing water for 2 h at room temperature and then incubated with a 30% sucrose solution in PBS at 4 °C for at least 2 d. A microtome (Leica CM 1850) was used to coronally section (35 µm) the brain mPFC. The sections were mounted with Vectashield mounting medium containing DAPI (Vector Laboratories Inc.), and visualized using a confocal laser scanning system (Leica DM IRE2).

Behavioral examinations.

Open field test (OFT). The open field apparatus consisted of a rectangular chamber (40 × 40 × 30 cm) which was made of gray polyvinyl chloride. The mice were gently placed at the center and were allowed to explore the area for 30 min. The digitized image of the track taken by each mouse was stored, and the locomotion activity was analyzed post hoc using EthoVision 7.0 software.

Forced swim test (FST). Each mouse was placed in a clear glass cylinder (height 45 cm, diameter 19 cm), which was filled to 23 cm with water (22–25 °C). Mice were forced to swim for 6 min. The immobility time (minimal movements to keep the head above water) during the final 4 min were calculated by an investigator who was blind to the study conditions.

Tail suspension test (TST). Each mouse was hung 50 cm above the floor for 6 min by adhesive tape at approximately 1 cm from the tip of the tail. The mice were considered as immobile during 6 min test period, which can be defined as motionless hanging without any struggling behaviors. Any mice that did climb their tails were excluded from further experimental analysis.

Chronic social defeat stress (CSDS). CSDS was performed as described previously [19]. Briefly, a singly caged 8-10 weeks old male C57BL/6J mouse (test mouse) was exposed to a CD1 aggressor mouse (target mouse) for 10 min each day for a total of 10 d. To separate the susceptible and resilient subpopulations, the avoidance behaviors were tested 24 h following the final session, and a social interaction ratio (time spent in the interaction zone in the presence versus the absence of a target mouse) of 1.0 was set as a cutoff. The mice with scores <1.0 were considered susceptible, and those with scores ≥1.0 were considered resilient. The duration of time that the mice spent in the interaction zone was obtained using EthoVision 7.0 software (Noldus).

In the drug treatment experiment, all of the mice were housed individually. Following the CSDS protocol, the defeated mice were randomly divided into three groups and were given daily either saline, rosiglitazone (5 mg/kg, i.p.) or imipramine (15 mg/kg, i.p.) for 10 consecutive days. The long-term behavioral consequences of defeat stress were assessed using the social avoidance test 24 h following the final injection.

Sucrose preference test (SPT). The procedure of sucrose preference test was performed as previously described, with modified [20,21]. Mice were singly caged and acclimated to drink from two 50-ml bottles (A and B) for 4 days. One of which was contained with 1% sucrose and the other with water. The sucrose preference was determined for 2 h after a 20 h period of water and food deprivation. The sucrose preference for each mouse was calculated as $100 \times (\text{VolA} / (\text{VolA} + \text{VolB}))$, and the total fluid intake was calculated as $\text{VolA} + \text{VolB}$.

Western blot analysis. Mice were sacrificed, and brains of which were removed and coronally sliced according to the brain matrices; PFC, mPFC and hippocampus tissues were rapidly dissected and frozen on dry ice. For protein extractions, all tissue samples were homogenized in RIPA-buffer with a protein protease inhibitor cocktail. The membranes were incubated with PPAR γ (1:1,000, Santa Cruz, California, USA) or GAPDH (1:4,000, Proteintech, Wuhan, China) antibodies and were visualized using chemiluminescence detection solutions (PerkinElmer). The FluorChemTM SP software was used to measure band densitometry.

Statistical analysis. Data are shown as the mean $\pm$ SEM. Student's t-test was applied for comparisons between 2 groups, and one-way and two-way ANOVA were used to compare 3 or more sets of data, followed by Tukey's multiple comparisons test or Sidak's multiple comparison test separately, using SPSS 20.0 software. A probability (p) value of <0.05 was considered to be statistically significant.

Results

A long-term HFD impaired glucose tolerance and increased depressive-like behaviors.

To generate obese mice, we subjected 8-week-old male C57BL/6J mice to the HFD feeding regimen for 16 consecutive weeks (HFD, 60% kcal fat, D12492, Research Diets Inc.), with the control mice being fed the control diet (CD, 10% kcal fat, D12450B, Research Diets Inc.) (Fig. 1A). Two-way repeated measures of ANOVA revealed that HFD significantly increased the body weight of mice (interaction, $F_{(1, 44)} = 37.86$, $p < 0.0001$). No difference of body weight was found between the two groups before diet manipulation (CD 21.3 ± 0.4 g, HFD 21.1 ± 0.5 g; $p = 0.9692$, Sidak's multiple comparisons test). The 16-week HFD feeding regimen resulted in a 34% increase in body weight compared with the CD feeding regimen (CD 27.6 ± 0.6 g, HFD 37.1 ± 1.4 g; $p < 0.0001$, Sidak's multiple comparisons test) (Fig. 1B). Glucose tolerance impairment also arose in HFD-fed mice (interaction $F_{(4, 40)} = 12$, $p < 0.0001$), with a tendency of increase (CD 4.6 ± 0.2 mmol/l, HFD 6.2 ± 0.5 mmol/l; $p = 0.0736$, Holm-Sidak's multiple comparisons test) and a deficit in controlling blood glucose level after sugar intake (30 min: CD 8.9 ± 0.5 mmol/l, HFD 11.1 ± 0.8 mmol/l; $p < 0.05$, 120 min: CD 5.8 ± 0.6 mmol/l, HFD 15.0 ± 0.9 mmol/l; $p < 0.0001$, Holm-Sidak's multiple comparisons test) (Fig. 1C).

To assess the effects of a long-term HFD feeding regimen on depressive-like behaviors, we subjected both groups of mice to a series of specific behavioral tests, including the OFT, the SPT, the TST and the FST (Fig. 1A). After 16 consecutive weeks of feeding, the HFD-fed group showed a significant reduction in

sucrose consumption ratio compared to the CD-fed group (Fig. 1D, $p<0.05$). The total intake of water and sucrose consumption was similar between the two groups, while the intake of sucrose solution was much higher in the HFD-fed mice compared with the CD-fed mice (As shown in **Supplementary Fig. 1**). The results indicated the presence of anhedonic behaviors in the HFD-fed group. The immobility time of the HFD-fed group increased in the TST (Fig. 1E, $p<0.01$) and FST (Fig. 1F, $p<0.05$) with no significant difference found between two groups in OFT (Fig. 1G, $p>0.05$), which was consistent with the results of a previous study [22]. These results indicated that a chronic HFD feeding regimen led to depressive-like behaviors without a change in spontaneous activity.

PPAR γ expression decreased in brain of HFD-fed mice.

To determine whether brain PPAR γ might be related to HFD-induced depressive-like behaviors, we first measured PPAR γ protein levels in the brain areas correlating with depression, namely, the PFC, mPFC and hippocampus. Compared to CD-fed mice, HFD-fed mice showed a decreased level of PPAR γ protein in the mPFC and PFC but not in the hippocampus (Fig. 1H and 1I).

Neuronal PPAR γ knockout mice displayed depressive-like behaviors.

Given that PPAR γ expression decreased in brain of HFD-fed mice, we generated neuronal-specific PPAR γ knockout mice (NKO) using the Cre-LoxP recombination system to determine whether neuronal PPAR γ deficiency was correlated with depressive-like behaviors. To achieve neuronal selectivity for PPAR γ deletion, we crossed PPAR $\gamma^{fl/fl}$ mice with mice expressing Cre under a well-characterized CaMKII α promoter to drive Cre expression. The littermate PPAR $\gamma^{fl/fl}$ (LoxP) mice were used as controls. As the PPAR γ was observed in both neuronal and glial cells [23], to exclude the impact of glial cells, we checked PPAR γ expression in primary cultured cortical neurons from both NKO and LoxP mice. As shown in Fig. 2A and Fig. 2B, on day 15, there was a significant loss of PPAR γ protein expression in the cultured neurons from the NKO mice compared with those from the LoxP mice. The NKO mice had a normal appearance, and the neuronal PPAR γ deficiency did not affect animal growth or body weight (As shown in **Supplementary Fig. 2A and Fig. 2B**).

To determine whether neuronal-specific knockout of PPAR γ affected depressive-like behaviors, TST and FST were performed, in which the duration of immobility time increased in the NKO group comparing with the LoxP group (Fig. 2C and 2D, $p<0.05$), with no significant differences observed between groups in terms of the total distance traveled in the OFT (Fig. 2E, $p>0.05$). Moreover, the increased immobility time in the FST of the NKO mice were reversed by acute treatment with imipramine (15 mg/kg body weight, by intraperitoneal (i.p.) injection) ($F_{(2, 29)} = 4.655$, $p=0.0176$; one-way ANOVA) (Fig. 2F).

In the social interaction test, two-way repeated-measures ANOVA revealed that neuronal-specific knockout of PPAR γ significantly reduced the increased interaction time (interaction, $F_{(1, 27)} = 19.89$, $p=0.0001$). NKO mice showed no significant interest to target (no target 41.5 ± 3.3 s, target 38.7 ± 3.7 s, $p>0.05$), while LoxP mice had a longer interaction time when the target was present (no target 34.2 ± 3.15 s, target 55.9 ± 4.1 s,

$p < 0.001$) (Fig. 2G). These results indicated that neuronal PPAR γ deficiency induced depressive-like behaviors in adult mice.

Neuronal PPAR γ deficiency in mPFC caused depressive-like behaviors in mice.

Given that PPAR γ expression was decreased in the mPFC of HFD mice and that the PPAR γ -NKO mice showed increased depressive-like behaviors, we used an AAV-mediated approach to achieve neuronal knockout of PPAR γ in the mPFC to determine the contribution of neuronal PPAR γ deficiency in the mPFC to depressive-like behaviors. PAOV- CaMKIIa -EGFP-T2A-Cre (CaMKIIa-Cre) or pAOV- CaMKIIa-EGFP-T2A-3FLAG (CaMKIIa-EGFP) viruses were injected into the mPFC of LoxP mice (Fig. 2H). CaMKIIa-Cre infection induced PPAR γ knockout in pyramidal neurons and produced GFP to mark the infected neurons (Fig. 2H). Three weeks after the vector injections, depressive-like behaviors in the LoxP group were evaluated. We found that the immobility time increased in the CaMKIIa-Cre-LoxP group compared with the CaMKIIa-EGFP-LoxP group in the TST and FST (Fig. 2I and 2J, $p < 0.05$), with no change in the total distance in the OFT (Fig. 2K, $p > 0.05$), and these behaviors were equivalent to those of the NKO mice. Our results proved that neuronal PPAR γ deficiency in mPFC is sufficient to induce depressive-like behaviors in mice.

Neuronal PPAR γ overexpression in mPFC rescued depressive-like behaviors in NKO mice.

Next, we used a viral expression approach to examine whether neuronal-specific overexpression of PPAR γ in the mPFC is sufficient to rescue the depressive-like behaviors in NKO mice. To this end, we stereotactically injected the pAAV-syn-EGFP-T2A-PPAR γ -WPR-ES-pAS (AAV-PPAR γ) vectors to induce neuronal-specific overexpression of PPAR γ . The schematic of the AAV-PPAR γ vector is shown in Fig. 3A (top), and the pAAV-syn-EGFP-T2A-WPR-ES-pAS (AAV-EGFP) viruses were used as the control viruses. The injection locations of AAV were illustrated on a brain atlas image as shown in **Supplementary Fig. 3**. Western blotting revealed that the expression of PPAR γ increased significantly in the mPFC after 2 weeks of injection of the AAV-PPAR γ vectors (Fig. 3A, bottom). Next, we microinjected AAV-PPAR γ or AAV-EGFP vectors into the mPFC of LoxP and NKO mice. The timeline of the experimental procedure is shown in Fig. 3B (top). Either AAV- PPAR γ or AAV-EGFP was injected into the mPFC of NKO mice, and behaviors were examined 3 weeks later. We found that PPAR γ overexpression in the mPFC reversed the increased immobility time in the FST ($F_{(2,33)} = 4.305$, $p = 0.0218$) and TST ($F_{(2,32)} = 6.772$, $p = 0.0035$) (Fig. 3C and Fig. 3D), with no effect on the total distance in the OFT ($F_{(2,28)} = 1.882$, $p = 0.1710$) (Fig. 3E, $p > 0.05$), indicating that PPAR γ deficiency in the mPFC contributed to depressive-like behaviors in NKO mice.

Neuronal PPAR γ overexpression in mPFC blocked depressive-like behaviors in HFD mice.

Next, we tested whether neuronal PPAR γ overexpression could block the depressive-like behaviors in HFD mice. The timeline of the experimental procedure is shown in Fig. 3B (bottom). We found that neuronal-specific overexpression of PPAR γ significantly reduced the increased immobility time in FST (interaction, $F_{(1,42)} = 4.748$, $p = 0.035$). The immobility time reduced in HFD-fed mice (AAV-EGFP 124.1 ± 8.6 s, AAV-PPAR γ 84.9 ± 11.45 s; $p < 0.05$, Sidak's multiple comparisons test) but not in CD-fed mice (AAV-EGFP

94.3±12.4 s, AAV-PPAR γ 101.5±9.5s; $p>0.05$, Sidak's multiple comparisons test, Fig. 3F). Similar results were found in TST (interaction, $F_{(1,33)}=8.531$, $p=0.0063$), with immobility time reduced in HFD-fed mice (AAV-EGFP 94.3±12.4 s, AAV-PPAR γ 101.5±9.5 s; $p<0.05$, Sidak's multiple comparisons test) but not in CD-fed mice (AAV-EGFP 186.9±14.7 s, AAV-PPAR γ 219.1±7.7 s; $p>0.05$, Sidak's multiple comparisons test) (Fig. 3G). Meanwhile, no significant difference in the total distance was found in the OFT ($p>0.05$, Sidak's multiple comparisons test) (Fig. 3H). Our data indicated that neuronal PPAR γ deficiency in the mPFC of HFD mice is responsible for the depressive-like behaviors.

PPAR γ decreased in the mPFC of susceptible mice after chronic social defeat stress.

The CSDS model mimics several psychopathological dimensions of depression. In this study, mice were exposed to 10 days of repeated social defeat stress, and social withdrawal behaviors were subsequently analyzed using a social interaction test 24 h after the last defeat episode. Social interaction ratio is the time spent in the interaction zone with the target present divided by the time spent in the interaction zone with the target absent. Usually, social interaction ratio equaling to 1.0 is used as the threshold for dividing defeated mice into susceptible and resilient groups (Fig. 4A). Two-way repeated-measures ANOVA showed a response pattern difference among mice in social interaction test after a 10-day process of CSDS (interaction: $F_{(2,68)} = 22.92$, $p<0.0001$). Susceptible mice spent significantly less time in the interaction zone than the control or resilient mice when a target was present (target: control mice 73.3±3.6 s, susceptible mice 35.2±6.0 s, resilient mice, 86.6±2.8 s; Sidak's multiple comparisons test), while no difference was shown among groups when no target existed (no target: control mice 59.1±3.0 s, susceptible mice 74.4±3.1 s, resilient mice, 64.8±3.8 s; Sidak's multiple comparisons test) (Fig. 4B).

Next, we investigated whether PPAR γ expression changed in the mPFC of the CSDS mice. At 48 h after the final defeat episode, susceptible mice showed less PPAR γ protein in their mPFC than either the resilient or control mice, and the resilient mice showed increased PPAR γ protein in their mPFC compared with the control mice, whereas the PPAR γ protein levels in the PFC and hippocampus were comparable among the subgroups (Fig. 4C and 4D). We found a significant positive correlation between the PPAR γ protein and social interaction ratio (Fig. 4E, Pearson correlation, $r = 0.646$, $p<0.05$).

Previous studies showed that socially defeated mice demonstrate long-lasting social avoidance that is reversed by chronic (but not acute) treatment with antidepressants [2]. To determine whether rosiglitazone could reverse social withdrawal behaviors, after 10 days of social defeat stress, the defeated mice were treated with saline, rosiglitazone (5 mg/kg) or imipramine (15 mg/kg) for 10 consecutive days by i.p. injection. Two-way repeated-measures ANOVA (interaction, $F_{(3,57)} = 6.464$, $p=0.0008$) showed that a 10-day treatment of rosiglitazone was sufficient to stably reverse the avoidance behaviors of defeated mice (target, saline 35.8±4.3 s, rosiglitazone 61.7±7.6 s, $p<0.05$, Holm-Sidak's multiple comparisons test). Meanwhile, no difference was found among treatments while no target was introduced ($p>0.05$, Holm-Sidak's multiple comparisons test) (Fig. 4F). Previous reports have shown that a 4-week treatment with the well-characterized antidepressant imipramine is necessary to increase the interaction time of defeated mice to the levels of the control mice [24], whereas a 10-day

treatment with imipramine is ineffective, which is consistent with our results. Taken together, these results indicate that PPAR γ decreases in the mPFC of susceptible mice, and increased in the mPFC of resilient mice after CSDS and that the agonist of PPAR γ alleviates CSDS-induced depressive-like behaviors.

Discussion

In this study, we explored the role of PPAR γ in depressive-like behaviors. We demonstrated that HFD induced PPAR γ impairment in the brain leading to depression. First, we found that PPAR γ decreased in mPFC of HFD-fed mice (Fig. 1). Because PPAR γ is found in astrocytes, neurons and cerebrovascular endothelial cells, PPAR γ of which cell might be involved in depression? We further proved that neuronal-specific PPAR γ knockout was sufficient to induce depressive-like behaviors, and mPFC neuronal-specific PPAR γ deficiency mice showed similar behavior (Fig. 2 and Fig. 3), suggesting that PPAR γ impairment in mPFC neurons caused depression. More importantly, making up for PPAR γ decrease in the mPFC neurons caused by HFD reversed the depressive-like behaviors of HFD-fed mice (Fig. 1 and Fig. 3). Our data proved that PPAR γ dysfunction in the mPFC neurons might be the main causes of depression in HFD mice. Meanwhile, we found that PPAR γ expression was decreased in the mPFC of susceptible mice, and increased in the mPFC of resistant mice after CSDS (Fig. 4), and a 10-day treatment with PPAR γ agonist rosiglitazone blocked these depression-related behaviors. Our data suggested that PPAR γ also participated in the pathophysiology of social defeat-induced depression.

HFD induced PPAR γ dysfunction is involved in the pathogenesis of metabolic disorders. For example, HFD induces cyclin-dependent kinase 5 (CDK5) dependent PPAR γ phosphorylation, which gives rise to dysregulated expression of a subset of genes including a number of key metabolic regulators, resulting in insulin resistance [10]. In addition, HFD disrupts the normal circadian cycle and causes a reprogramming of the metabolic and transcriptional liver pathways, partly mediated by inducing the *de novo* oscillation of PPAR γ -mediated transcriptional control at otherwise non-cyclic genes [25]. In this study, we found HFD induced PPAR γ dysfunction in brain mediating depressive like behaviors. Taken together, HFD not only induces peripheral PPAR γ dysfunction mediating somatic manifestations, but also induces central PPAR γ dysfunction mediating affective components of depression. Recently, varieties of evidences implicated that depression is a systemic disease with multiple physical symptoms rather than a mental illness. The above findings provide clues to understand why depression frequently occurs comorbidly with diabetes.

As to the high abundance and important role of PPAR γ in white fat tissue, studies explored the mechanism how rosiglitazone exerts antidepressant effect in adipose tissue, and found that it elicited antidepressant effect through the PPAR γ -dependent induction of adiponectin [26,27]. However, the finding does not rule out the possibility that brain PPAR γ exerts antidepressant effects. In our study, mPFC neuronal-specific PPAR γ knockout is sufficient to induce depressive behaviors, and overexpression of PPAR γ in mPFC neurons can rescue the depressive behaviors of HFD-fed mice. The literature and our study suggest that PPAR γ might play anti-depressant effect by both peripheral and central signaling activation.

In neurons, PPAR γ regulates a series of key factors which have been reported to be involved in depression. PPAR γ activation in hippocampal neuronal cells increases brain-derived neurotrophic factor (BDNF) expression, a key modulator in synaptic plasticity and depression [28]. PPAR γ was also found to bind to the promoter of neurotrophic factor- α 1 and upregulate its expression in neurons [29]. Neurotrophic factor- α 1 has been shown to mediate its antidepressant effects through fibroblast growth factor 2 (FGF2) and neurogenesis [30]. Furthermore, mass spectrometry and bioinformatics analysis revealed that PPAR γ agonists may induce the expression of synaptic vesicle-related genes, such as vesicle associated membrane protein 2 (VAMP2) and syntaxin binding protein 5 (STXBP5) [31], and the loss of synapses has been observed in depression. Neuronal PPAR γ dysfunction may result in the abnormal expression of diverse neurotrophic factors and synapse-related genes, leading to transmitter release disorders and behavioral abnormalities. In addition, it is reported that PPAR γ agonist rosiglitazone reduces corticotroph cell number, proopiomelanocortin (POMC), adrenocorticotrophic hormone (ACTH) and glucocorticoid receptors (GR) content in pituitary of diabetic rats [32]. PPAR γ might also work on hypothalamus-pituitary-adrenal (HPA) axis to play antidepressant effect.

PPAR γ signaling is well known for its role in inflammation and oxidative stress [33], both implicated in the pathophysiology of depression[34]. Due to the important roles of microglia and astrocytes in brain inflammation and oxidative stress, even our study suggested neuronal PPAR γ dysfunction in mPFC leads to depression, it does not exclude the possibility that PPAR γ signaling in astrocytes or microglia is involved in antidepressant effects.

Abbreviations

PPAR γ : peroxisome proliferator-activated receptor gamma; CNS: central nervous system; FST: forced swim test; TST: tail suspension test; HFD: high-fat diet; mPFC: medial prefrontal cortex; CaMKII α : calcium/calmodulin dependent protein kinase II α ; GTT: glucose tolerance test; CSDS: chronic social defeat stress; SPT: sucrose preference test; CDK5: cyclin-dependent kinase 5; FGF2: fibroblast growth factor 2; VAMP2: vesicle associated membrane protein 2; STXBP5: syntaxin binding protein 5; POMC: proopiomelanocortin; ACTH: adrenocorticotrophic hormone; GR: glucocorticoid receptor; HPA: hypothalamus-pituitary-adrenal; BDNF: brain-derived neurotrophic factor.

Declarations

Acknowledgements and Consent to participate

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Competing Interests

The authors declare no competing interests

Author Contributions

C.C. Fu: contributed to the conception of the study, performed the experiment, collected the data and wrote the manuscript; X.Y. Zhang: performed the data analyses;

L. Xu and S. Xu helped to produce the HFD-induced obese mice and conducted the behaviors test; Q.Q. Wu, Z. Wang, L.F. Lin and H.X. Huang contributed to produce and validate the PPAR $\gamma^{fl/fl}$ mice and neuronal-specific PPAR γ knockout mice; X. Gu, S. Lin and Y.Y. Fang helped with the data collection and experiments preparation; X. Cao helped designed the study; X.M. Wang designed the study, revised the manuscript and oversaw the entire project.

Data Availability

All datasets generated and analyzed during this study are included in this published article and its supplementary information files. Materials are available upon request.

Ethical Approval

All experiments animals were performed with the approval of the Southern Medical University Animal Ethics Committee.

Consent to participate

Not applicable.

Consent to publish

All authors have given consent for publication.

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Figures

Figure 1

A long-term HFD impaired glucose tolerance and increased depressive-like behaviors. A. Schematic diagram of the CD/HFD feeding and behavior tests design. B. The initial and final body weights of male C57BL/6J mice after 16 weeks of HFD feeding. $n=12$ mice per group, $***p<0.001$. C. Blood glucose levels after D-glucose (2 g/kg) injection in mice fed the CD or HFD, $n=5$ mice per group. D. The percentage of sucrose consumption in the sucrose preference test, $n=14-15$ mice per group. E-F. The effect of the chronic HFD consumption on depression-like behaviors expressed as the immobility time in the TST and FST (E and F), $n=14-15$ mice per group. G. The effect of chronic HFD consumption on spontaneous locomotion, as assessed by the OFT, $n=12-13$ mice per group. H. Western blot images and quantification of PPAR γ levels in the PFC, mPFC and hippocampus of the two groups of mice following 16 weeks with the CD or HFD. $n= 6-8$ mice per group. I. Densitometry values for PPAR γ were normalized to those of GAPDH. Data are the means $\pm$ SEMs. $*p<0.05$, $**p<0.01$ and $***p<0.001$.

Figure 2

Neuronal PPAR γ knockout mice displayed depressive-like behaviors. A. Western blot showing PPAR γ and GAPDH protein levels in cultured neurons at day 15 in culture that were generated from NKO and control (LoxP) mice. B. The quantification of PPAR γ levels in NKO and LoxP mice. $n = 3$ mice per group. C. The immobility time increased for the NKO group compared with the LoxP group in the FST, $n = 12$ mice per group. D. The immobility time increased for the NKO group compared with the LoxP group in the TST,

n=11 mice per group. E. The influence of neuronal PPAR γ deficiency on spontaneous locomotion, as assessed in the open field test, n=8-10 mice per group. F. The increased immobility time of the NKO group is reversed by imipramine in the FST, n=10-11 mice per group. G. The avoidance behaviors of the NKO mice in the social interaction test, n=14-15 mice per group, **p< 0.001, two-way repeated-measures ANOVA (interaction, F (1, 27) = 19.89, p=0.0001) followed by Sidak's multiple comparison test. H. Confocal images showed the injection site of the AAV-CaMKII α -Cre vector into the mPFC to induce PPAR γ knockout in pyramidal neurons and produced GFP to mark the infected neurons. I-J. PPAR γ knockout in the mPFC of LoxP mice with AAV-CaMKII α -Cre increased depression-like behaviors, as reflected by increased immobility time in the FST and TST, n=9-11 mice per group. K. PPAR γ knockout in mPFC of LoxP mice with AAV-CaMKII α -Cre had no effect on locomotive activity in the open field test, n=9-11 mice per group. Data are the means $\pm$ SEMs. *p<0.05, **p<0.01 and ***p<0.001.

Figure 3

Neuronal PPAR γ overexpression in mPFC rescued depressive-like behaviors in NK0 and HFD mice. A. Schematic of the pAAV-syn-PPAR γ vector for overexpression of PPAR γ (top). Western blots show PPAR γ overexpression in the mPFC injected with AAV-syn-PPAR γ vector (bottom). B. Timeline of microinjections of pAAV-syn-PPAR γ vector in LoxP and NK0 mice (top). Timeline of microinjections of pAAV-syn-PPAR γ vector in CD and HFD mice (bottom). C-D. Neuronal-specific overexpression of PPAR γ from the mPFC

decreased the immobility time of the NKO mice in the FST and TST, n=12-14 mice per group, one-way ANOVA followed by Tukey's multiple comparisons test. E. Neuronal-specific overexpression of PPAR γ had no effect on spontaneous locomotion in the NKO group, n=10-11 mice per group, one-way ANOVA followed by Tukey's multiple comparisons test. F. Neuronal-specific overexpression of PPAR γ reduced the immobility time of the HFD-fed mice in FST. n=11-12 per group; *p< 0.05. Two-way ANOVA (interaction, F(1,42) =4.748, p < 0.05) followed by Sidak's multiple comparisons test. G. Neuronal-specific overexpression of PPAR γ in the mPFC decreased the immobility time of the HFD-fed mice in TST, n=11-12 mice per group. H. Neuronal-specific overexpression of PPAR γ had no effect on spontaneous locomotion in the HFD-fed group, n= 8-14 mice per group. Two-way ANOVA (interaction, F(1, 42)=1.635, p $\geq$ 0.05) followed by Sidak's multiple comparisons test. Data are the means $\pm$ SEMs. *p<0.05, **p<0.01 and ***p<0.001.

Figure 4

PPAR γ decreased in the mPFC of susceptible mice after chronic social defeat stress. A. Horizontal scatter plot illustrating the distribution of the interaction ratios of the control, susceptible, and resilient mice following the CSDS protocol, $n=10-16$ mice per group. B. Time spent in the interaction zone after 24 h of chronic defeat stress for the 3 subgroups, $n=10-16$ mice per group. C-D. Western blot images and quantification of PPAR γ levels in the PFC, mPFC and hippocampus of the three groups of mice following

CSDS, n=6 per group. E. Correlation of PPAR γ protein level in mPFC with social interaction ratio after CSDS (Pearson correlation, $r=0.646$, $p<0.05$). F. The avoidance behaviors of the mice after 10 days of treatment with saline, imipramine or rosiglitazone (i.p.) following the CSDS. A 10-day treatment with rosiglitazone stably reversed the avoidance behaviors of defeated mice. n=14-17 mice per group. Two-way ANOVA (interaction, $F(3,57) = 6.464$, $p=0.0008$) followed by Holm-Sidak's multiple comparisons test. Data are the means $\pm$ SEMs. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

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