Palmitoylethanolamide dampens neuroinflammation and anxiety-like behavior in obese mice

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Materials and Methods

Evaluation of metabolic parameters.

During the experimental period, body weight was weekly assessed. At the end of the experimental protocol, before sacrifice, bioelectrical impedance analysis was performed to determine fat body composition assessment using BIA 101 analyzer, modified for the mouse (Akern, Florence, Italy). The energy intake was calculated according to the energy content of the diets. Standard and HFD contained 15.8 and 21.9 kJ/g, respectively.

Measurement by LC-APCI-MS of PEA levels

Serum and tissues (hippocampus and hypothalamus) were homogenized in a solution of chloroform/methanol/Tris-HCl 50 mM pH 7.4 (2:1:1 by vol.) containing 10 pmol of [2H]4-PEA as internal standard (Bisogno et al., 1997; Petrosino et al., 2016). The lipid-containing organic phase was pre-purified by open-bed chromatography on silica gel, and fractions obtained by eluting the column with a solution of chloroform/methanol (90:10 by vol.) were analyzed by Liquid Chromatography-Atmospheric Pressure Chemical Ionization-Mass Spectrometry (LC-APCI-MS) by using a Shimadzu HPLC apparatus (LC-10ADVP) coupled to a Shimadzu (LCMS-2020) quadrupole MS via a Shimadzu APCI interface. LC-APCI-MS analysis of [2H]4-PEA was carried out in the selected ion monitoring (SIM) mode (Di Marzo et al., 2001; Marsicano et al., 2002), using m/z values of 304 and 300 (molecular ions + 1 for deuterated and undeuterated PEA, respectively). PEA levels were calculated based on their area ratio with the internal standard signal areas, and the amounts (pmol) were normalized per ml of volume or mg of tissue.

Statistical analysis

Data are presented as mean ± SEM. Analysis of body weight, fat mass and energy expenditure between STD and STD+PEA groups was performed using the Student's t-test. The evaluation of PEA levels was analyzed using analysis of variance (ANOVA) for multiple comparisons followed by Bonferroni's post hoc test, using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). Normality was tested using Shapiro-Wilk test. Differences among groups were considered significant at values of $p < 0.05$.

Supplementary Figures

Supplementary Figure S1

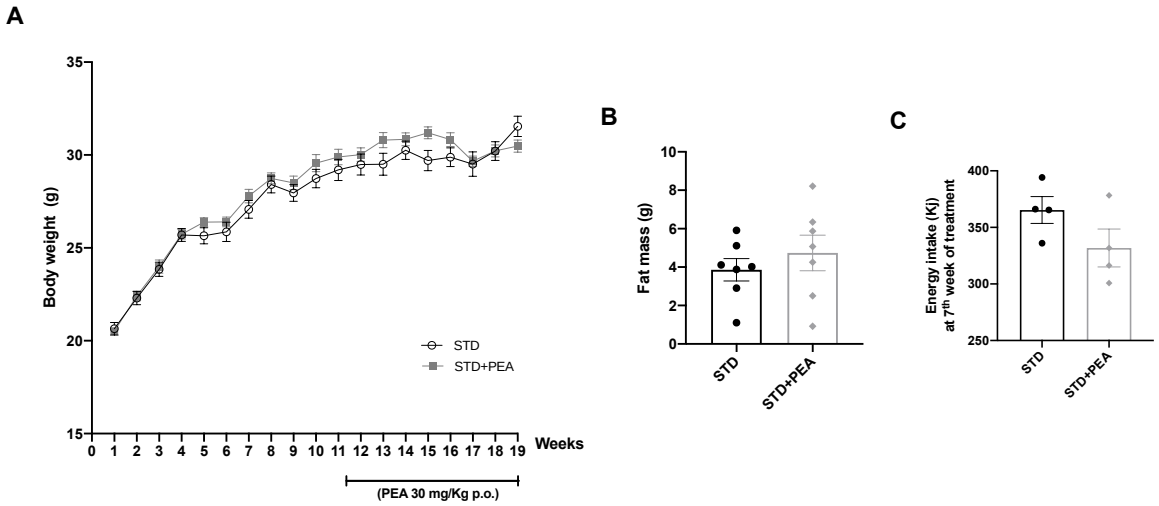

Figure S1

Supplementary Figure S1. Effect of 7-week PEA treatment on metabolic parameters in STD chow diet fed mice. (A) The body weight of vehicle- (STD) and PEA-treated (STD+PEA) animals was monitored for all experimental time (n=10). At 19th week, (B) fat mass and (C) energy intake (15.8 kJ/g for each gram of chow diet, measured for each cage with 4-5 animals) were also evaluated (for fat mass n=7, and for energy intake n=4). No significant modification was shown in PEA-treated mice in all metabolic parameters compared to untreated STD mice (for fat mass: $p = 0.4392$ and for energy intake: $p = 0.1521$). All data are shown as mean ± S.E.M.

Supplementary Figure S2

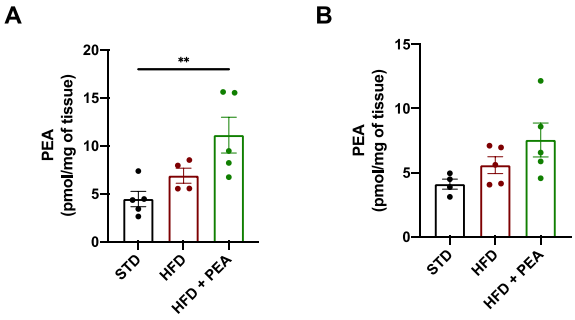

Figure S2

Supplementary Figure S2. PEA treatment induced an increase of its levels in brain areas of obese mice. mice. PEA levels in (A) hypothalamus and (B) hippocampus in all groups (for hypothalamus, STD: n=5, HFD: n=4, HFD+PEA: n=5, F (2, 11) = 1.896; STD: 4.452 ± 0.802; HFD: 6.890 ± 0.785, p = 0.4407 *vs* STD; HFD+PEA: 11.120 ± 1.872; p = 0.0093 *vs* STD p = 0.1147 *vs* HFD; for hippocampus, STD: n=4, HFD: n=5, HFD+PEA: n=5, F (2, 11) = 1.294; STD: 4.110 ± 0.396; HFD: 5.584 ± 0.656, p = 0.5441 *vs* STD; HFD+PEA: 7.554 ± 1.321; p = 0.0670 *vs* STD p = 0.3136 *vs* HFD). All data are shown as mean ± S.E.M. ***p* < 0.01.

Supplementary references

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