Supplementary Material

Materials and Methods

Echocardiography

Prior to initiation of the study, the mice were trained on two separate occasions over 1–2 days. Under electrocardiographic monitoring of heart rate (HR, bpm), the hearts were imaged in 2D long-axis views at the level of the highest left ventricular (LV) diameter. LV end-diastolic (LVIDd, mm) and end-systolic (LVIDs, mm) internal diameters, end-diastolic wall thickness of both interventricular septum (IVS, mm) and LV posterior wall (LVPW, mm), LV ejection fraction (EF, %) and fractional shortening (FS, %) were measured using M-mode in the parasternal long-axis view. Relative wall thickness (RWT), a measure of hypertrophy, was calculated as IVSd+LVPWd/LVID (1).

Pressure-volume loops

Briefly, mice were anesthetized and mechanically ventilated through tracheostomy with 6–7 μL/g tidal volume and 130 breaths/min under isoflurane. Blood volume expansion (12.5% human albumin, 50– 100 μL over 5 min) was provided through a 30-gauge cannula via the right external jugular vein. The LV apex was exposed through an incision between the seventh and eighth ribs, and a 1.4-Fr PV catheter (SPR 839; Millar Instruments Inc.) was advanced through the apex to lie along the longitudinal axis. Absolute left ventricular volume was calibrated, and PV data were measured at steady state and during transient reduction of venous return by occluding the inferior vena cava with a 6-0 silk snare suture. From the 10–15 successive cardiac cycles during the inferior vena cava occlusion, the end-systolic PV relation slope was derived.)

Electron paramagnetic resonance

Briefly, ~20 mg of tissue was homogenized in phosphatebuffered saline (PBS) containing 0.1 mMdiethylene-triaminepentaacetic acid (DTPA) and protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN) at pH 7.4. Nonsoluble fractions were removed by centrifugation at 15,000 g for 10 min at 4 oC. The homogenates were

kept on ice and analyzed immediately. Stock solutions of 1- hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine hydrochloride (CMH; Enzo Life Sciences, Farmingdale, NY) were prepared daily in nitrogen purged 0.9% (w/v) NaCl, 25 g/L Chelex 100 (Bio-Rad) and 0.1 mM DTPA and kept on ice. The samples were treated with 1 mM CMH at 37 oC for 2 min, transferred to 50-µl glass capillary tubes, and analyzed on a Bruker E-Scan (Billerica, MA) electron paramagnetic resonance (EPR) spectrometer at room temperature. EPR signal intensities were normalized with respect to the protein concentrations of the tissue homogenates as determined by Pierce BCA protein assay kit (Life Technologies).

Elevated plus maze

The test equipment consists of a four arms labyrinth two of which protected by lateral barriers (closed arms, 30 cm × 5 cm, × 15 cm) and the other two exposed (open arms, 30 cm × 5 cm, 350 lx). EPM exploits the rodent's conflict between aversion to open spaces (i.e. hides in closed arms of a labyrinth) and instinct to explore new environments (i.e. exploration of open arms of the same labyrinth). The experimental sessions have been videotaped by camera placed above the apparatus.)

Y-maze

(The test equipment consists of a Y-shaped labyrinth composed of three arms (30 cm × 5 cm × 15 cm). Y-maze test is based on the tendency of the mouse to explore new environments, hence preferring a labyrinth arm not yet explored to an already known one (2). The experimental sessions have been videotaped by camera placed above the apparatus.)

Histological analyses

All antibodies are listed in Supplementary Table 1.

Heart

Slides were obtained from FFPE blocks (5µm thick), then were deparaffinized with xylene, rehydrated with three consecutive ethanol washes (100%, 75%, and 30%) and two 1X PBS washes, underwent antigen retrieval (10mM

Tris 1mM EDTA, 10mins in pressure cooked), and blocked (5% BSA, 0.1% Triton-X, 1X PBS) for 1 hour at room temperature. Deparaffinized tissue sections were stained with antibodies shown in Supplementary Table 1. First, myocardium was incubated overnight at 4°C with mouse monoclonal antibody against cardiac troponin T (1:250; Thermo Fisher Scientific, Cat. No. MA5-12960). The following day, slides were washed three times with 1X PBS and then incubated with donkey anti-mouse Alexa Fluor 555 (1:250, Abcam, Cat.No. ab150106) for one hour at room temperature. Following three 1X PBS washes, slides underwent TUNEL staining using an in situ cell death detection kit according to manufacturer's protocol (Roche Applied Science, 12156792910). Following TUNEL staining, slides were washed three times with 1X PBS, then Fluoroshield with DAPI (Sigma, Cat. No. F6057) was added to slides and cover slipped. Immunoreactive signal was detected via laser scanning microscopy (Zeiss LSM 510 Meta). Sections were imaged on an Olympus BX51TF with a DP70 color camera (Olympus). Collagen was quantified in digitally acquired images (3). CSA was computed on cardiomyocytes in perfect transverse section with a centrally localized nucleus. To compute cardiomyocyte nuclear densities, 15-20 images per animal at 630X magnification were acquired and analyzed.

Hippocampus

Sections were cut in anteroposterior direction using a freezing microtome (Leica, Germany). Serial sections (one out of six) were selected to perform immunohistochemical staining of Parvalbumin (1:200, Synaptic System, Germany) and Glial fibrillary acidic protein (1:500 GFAP, Agilent, USA). Signal was revealed with 2 h incubation with, respectively, anti-guinea pig Alexa488 (1:500, Jackson Immunoresearch) and anti-rabbit Rhodamine Red X (1:500, Jackson Immunoresearch). For BrdU detection, free-floating sections were pretreated by denaturing DNA with 2 M HCl for 30 min at 37 °C . Slices were blocked for 1 h at room temperature (RT) with 10% normal goat serum (NGS), 0.3% Triton X-100 in PBS, and then incubated overnight at 4 °C with a rat monoclonal anti-BrdU antibody (1:200, Abcam, AB6326) diluted in NGS 5% and Triton 0.1% in PBS. Signal was finally revealed with 2 h incubation at RT with Cy3 conjugated anti-rat IgG (1:500, Jackson Immuno Research). We also performed a double labeling for BrdU and neuronal nuclei (NeuN, 1:1000, Millipore, revealed with AlexaFluor488 anti-guinea pig IgG (1:400, Jackson Immuno Research). Cells were counted on a fluorescence microscope (Zeiss) using a 20 × objective and the analysis was performed using Stereo Investigator software (Microbrightfield). All cells in the granule cell layer and subgranular zone of every sixth section were counted, and the resulting number of cells was multiplied by

6 to give an estimate of the total number of labeled cells. The quantification of GFAP expression was performed offline on images captured with a microscope (Axio Imager. Z2, Zeiss) equipped with Apotome.2 (Zeiss) with a 20 × EC-PLAN-NEOFLUAR objective. Z-series stacks were acquired with an interval of 1 µm for 3 slices per animal. Maximum intensity projections were produced and the analysis was performed using Fiji (ImageJ software). Images were first converted to 8 bit and 3 200 µm wide ROIs of DG, CA1 and CA2/3 were selected. For the 3 ROIs the mean grey value was calculated and normalized using the value taken from the same ROI in a control image acquired in the thalamus of the same slice were only background labeling was present. Normalized values of the 3 ROIs of the single animal were then averaged to obtain the mean GFAP expression value of the single animal. The total amount of GFAP staining was then given as the mean ±standard error for each experimental group.

References

1. Sebag IA, Gillis MA, Calderone A, Kasneci A, Meilleur M, Haddad R, et al. Sex hormone control of left ventricular structure/function: mechanistic insights using echocardiography, expression, and DNA methylation analyses in adult mice. Am J Physiol Heart Circ Physiol. 2011;301(4):H1706-15.

2. Maynard KR, Hill JL, Calcaterra NE, Palko ME, Kardian A, Paredes D, et al. Functional Role of BDNF Production from Unique Promoters in Aggression and Serotonin Signaling. Neuropsychopharmacology. 2016;41(8):1943-55.

3. Lionetti V, Matteucci M, Ribezzo M, Di Silvestre D, Brambilla F, Agostini S, et al. Regional mapping of myocardial hibernation phenotype in idiopathic end-stage dilated cardiomyopathy. J Cell Mol Med. 2014;18(3):396-414.

Supplementary Tables

Supplementary Table 1.

Antigen	Antibody (species)	Company	Antigen retrieval	Dilution	Incubation time and temperature	Secondary Antibody (fluorochrome and dilution)	Incubation time and temperature
Alpha-Sarcomeric Actin	Mouse monoclonal	Sigma #A2172	Citric buffer (pH6), 98°C, 40'	1:400	1h, 37°C	Cy5 1:400	1h, 37°C
Cardiac Troponin T	Mouse monoclonal	Thermo Fisher Scientific #MA5-12960	Citric buffer (pH6), 98°C, 40'	1:250	1h, 37°C	Alexa555 1:250	1h, 37°C
Glial fibrillary acidific protein (GFAP)	Rabbit polyclonal	DAKO #Z0334		1:500	O/N, 4°C	RRX 1:500	2h, RT
Parvalbumin	Guinea Pig polyclonal	Synaptic System, Germany #195 004		1:300	1h, 37°C	Alexa488 1:500	2h, RT
Smooth Muscolar Actin	Mouse monoclonal	DAKO #M0851	Citric buffer (pH6), 98°C, 40'	1:100	O/N, 4°C	Alexa488 1:600	1h, 37°C
Isolectin GS-IB4 Biotin conjugated	Molecular probe	Thermo Fisher Scientific #121414	Citric buffer (pH6), 98°C, 40'	1:200	O/N, 4°C	Streptavidin DyLight550 (Abcam ab 134348)1:200	1h, 37°C
Hoechst 33258		Thermo Fisher Scientific Cat# H3569		1:1000	3 min, RT		

Anti-BrDU	Rat polyclonal	AbCAM, ab152095	HCl 2MOL, 37°C, 30’	1:500	O/N, 4°C	CY3 1:500	2h, RT
Anti-NeuN	Guinea Pig polyclonal	MILLIPORE ABN90		1:1000	O/N, 4°C	Alexa488 1:400	2h, RT

Supplementary Figure Legends

Suppl. Figure 1. Representative scheme of resident intruder paradigm. (a) Resident CD1 mouse. (b) 10 minutes interaction among resident CD1 and intruderC57BL/6J mouse. (c) Resident and intruder separated by a perforated partition, which maintains them in continuous sensory contact.

Suppl. Figure 2. Number of attacks in mice fed with standard diet (Control) or high fat diet (HFD).

Suppl. Figure 3.PS in obese mice increases apoptosis of left ventricular (LV) cardiac cells. (a) Representative images of the detection of LV apoptotic cardiac cells in each experimental group after staining with cardiac troponin T.DAPI, 4′,6-diamidino-2-phenylindole; TUNEL,terminal deoxynucleotidyltransferasedUTP nick end labeling. (b) LV fraction of cardiac troponin positive cells that are TUNEL positive (%).All values are represented trough box-and-whisker plots (n=4-5 per group, two-way ANOVA, **p < 0.01 Vs Controls; # p<0.05 Vs Ob; $$p<0.01 Vs PS; &p<0.05 interaction between Ob and PS).

Suppl. Figure 4. Obesity and PS doesn't affect number of PV+ cells and volume of CA1 and CA2/3 regions but target an increased inflammatory state in all the hippocampal structures. Average measure of CA1 (a) and CA2/3 (b) volume quantified with Hoechst staining. Relative expression of GFAP in CA1 (c) and CA2/3 (d) hippocampal regions. Average number of PV+ interneurons in CA1 (e) and CA2/3 (f) regions. All values are represented trough box-and-whisker plots (n=4-5 per group, two-way ANOVA, **p < 0.01 and ***p < 0.001 Vs Controls; # p<0.05 and ## p < 0.01 Vs Ob; $$ p<0.01 Vs PS; &p<0.05 interaction between Ob and PS).

Suppl. Figure 5. Western blots assay of hippocampal tissues. Panel (a) and (b) show the full-length Western blots corresponding to cropped blots in the main text.

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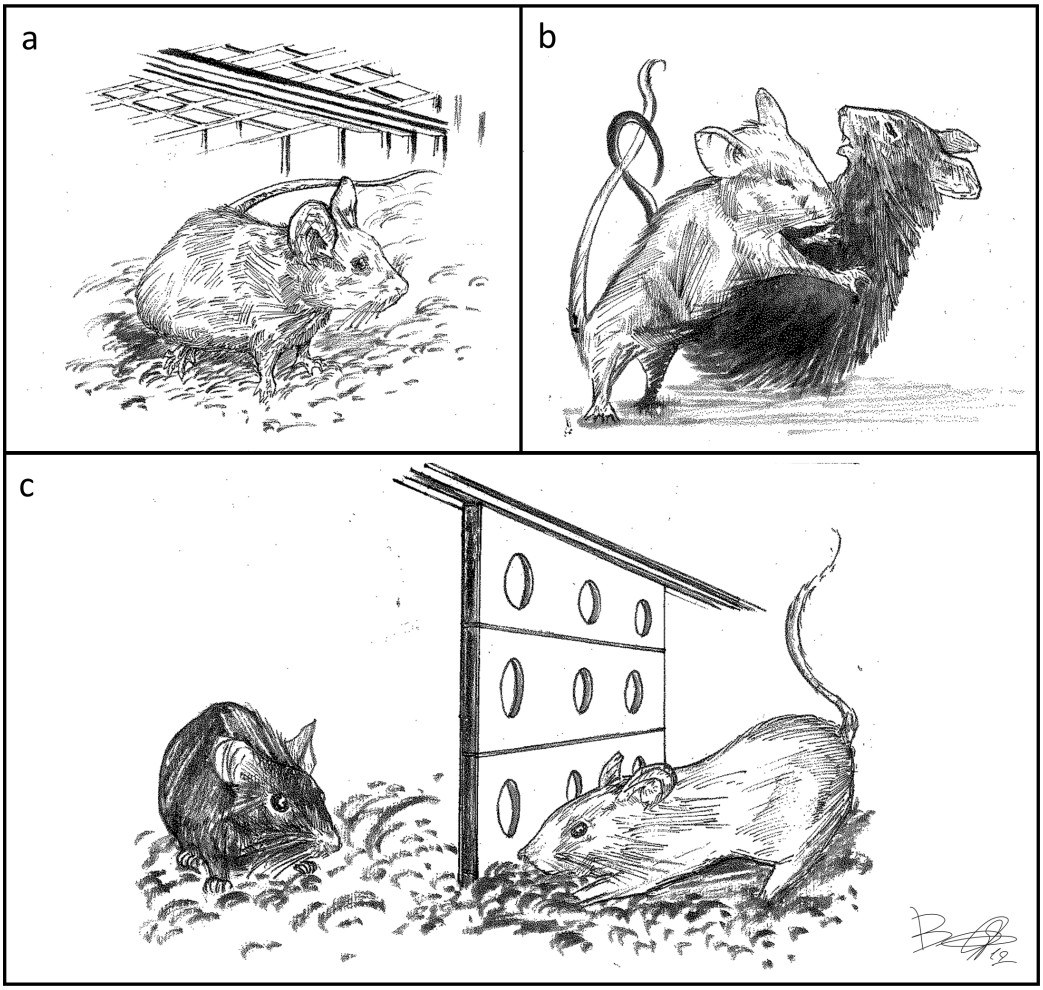
146 **Suppl. Figure 1.**



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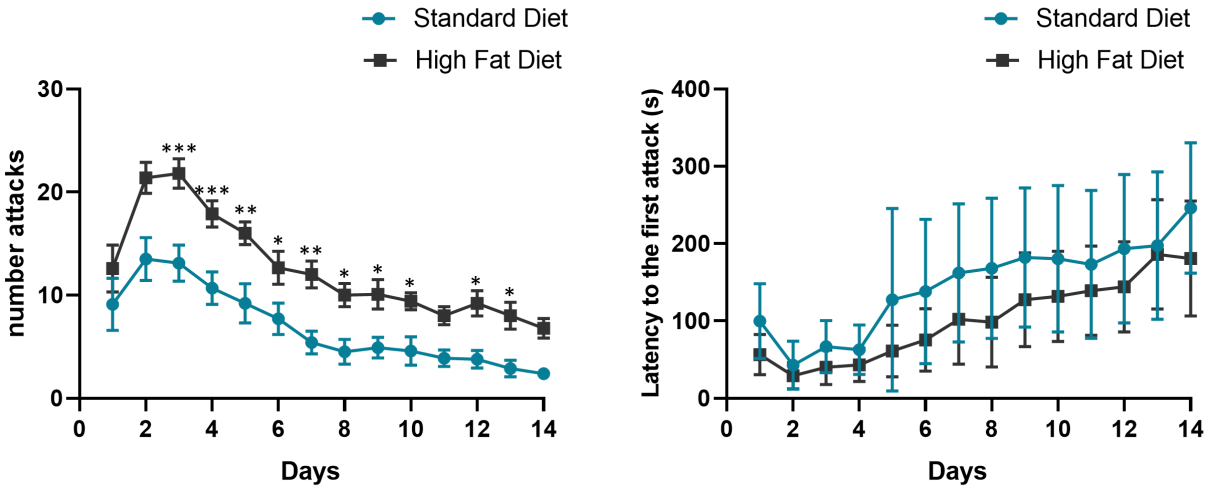
Suppl. Figure 2.



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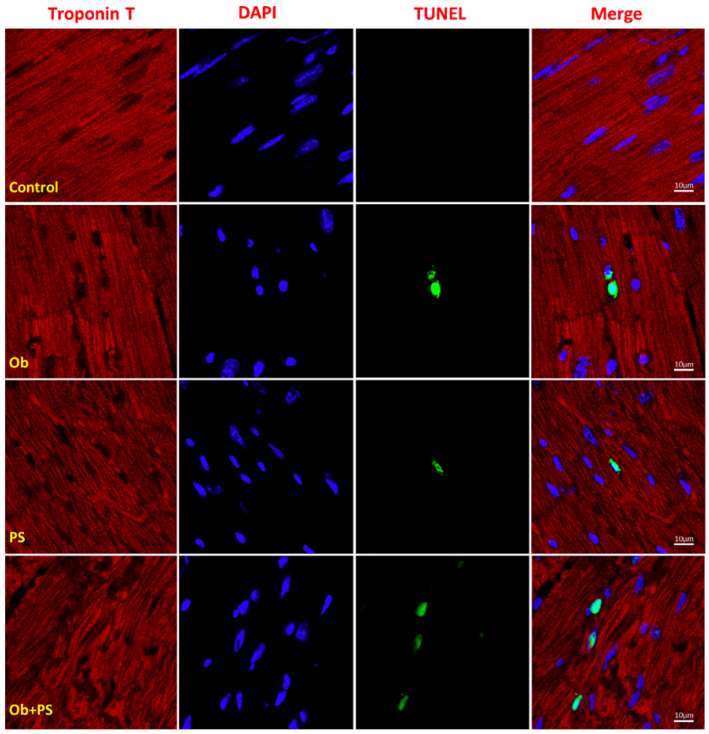
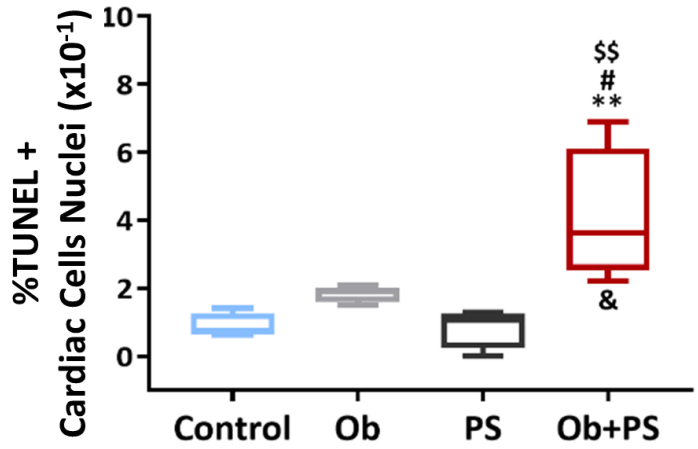
163 **Suppl. Figure 3.**





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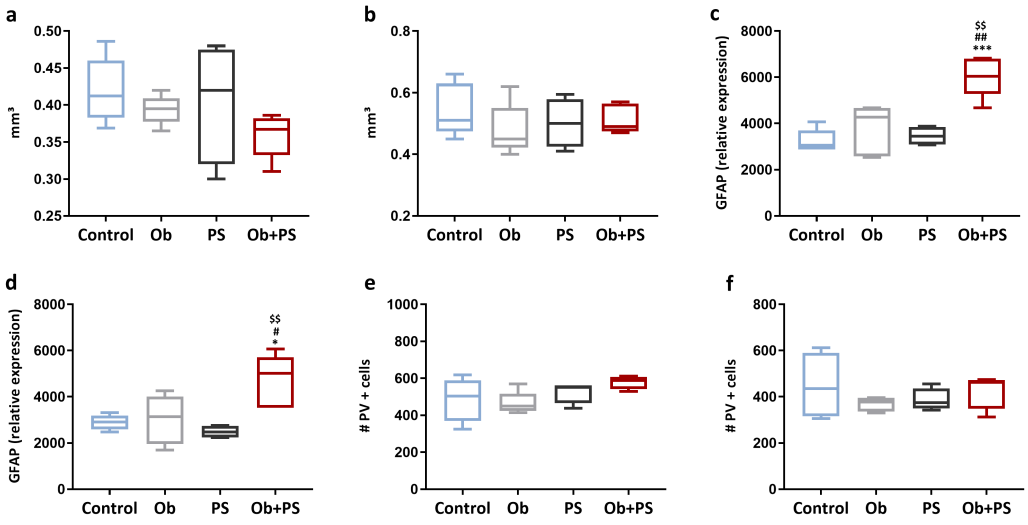
Suppl. Figure 4.



Suppl. Figure 5.