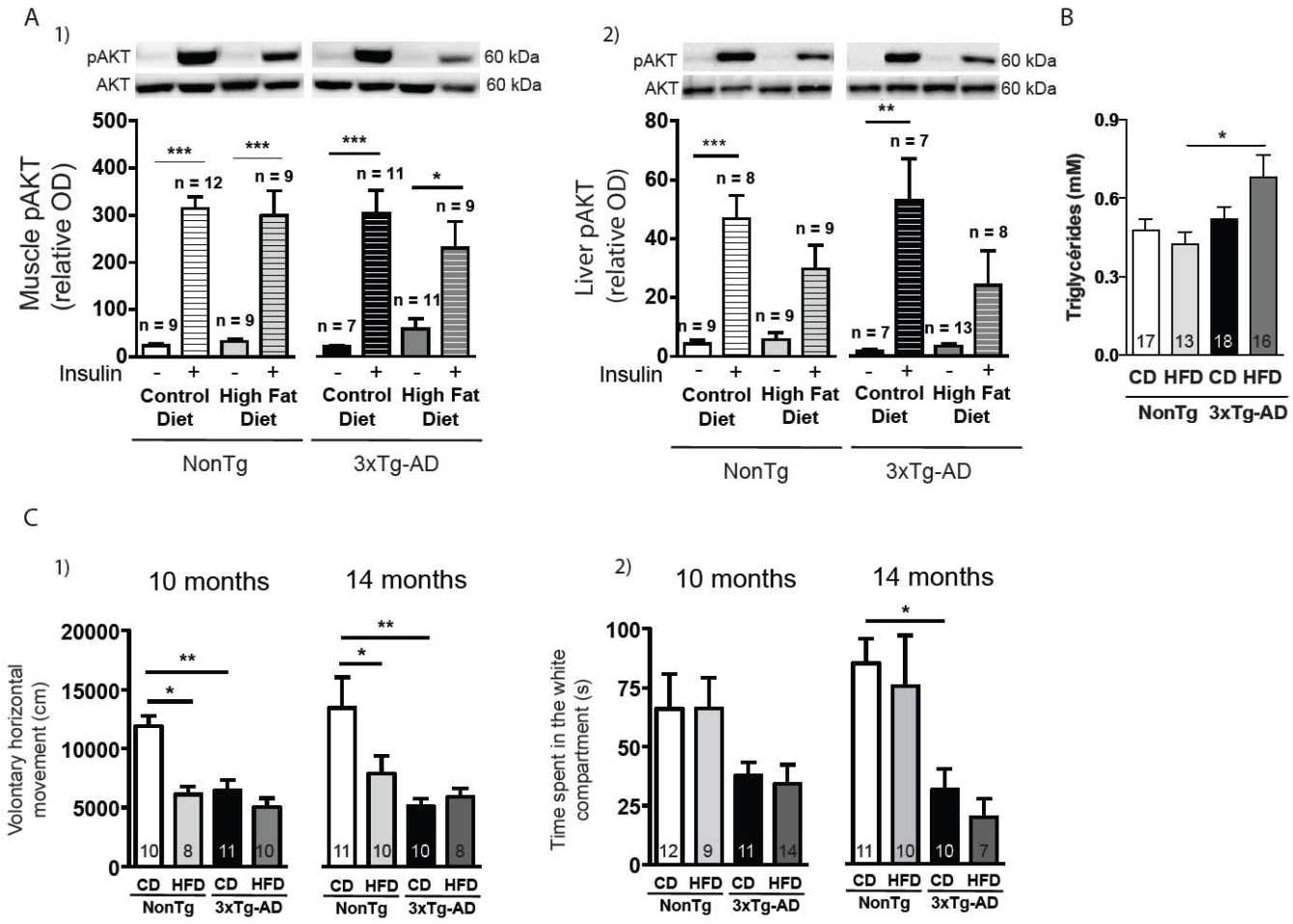
SUPPLEMENTARY DATA

Supplementary Figure 1. Effect of high-fat diet on peripheral metabolism and behavior of 3xTg-AD and NonTg mice. A) Reduced insulin sensitivity of 15-month-old mice following high fat diet ingestion. 1) Gastocnemius and 2) liver pAKT (pSer473) concentration measured by western blot 5 min. after I.V. insulin injection (3.8 U/kg). B) High fat diet increased plasmatic triglycerides 3xTg-AD mice. Triglycerides were measured in plasma sample of 15-month-old mice fed the control or the high fat diet for 9 months. C) High Fat Diet reduced locomotor activity in NonTg mice and 3xTg-AD mice had lower locomotor activity and higher anxiety-like behavior than NonTg mice. 1) Volontary horizontal movement was measured during a one-hour openfield session. 2) Anxiety-like behavior was evaluated using the dark-light emergence test.



Data are presented as mean ± SEM.
Data are compared using one-way ANOVA and Tukey's post-hoc analysis.
*p < 0.05, **p < 0.01, ***p<0.0001.
NonTg = Non-Transgenic mice, 3xTg-AD = triple-transgenic mice.

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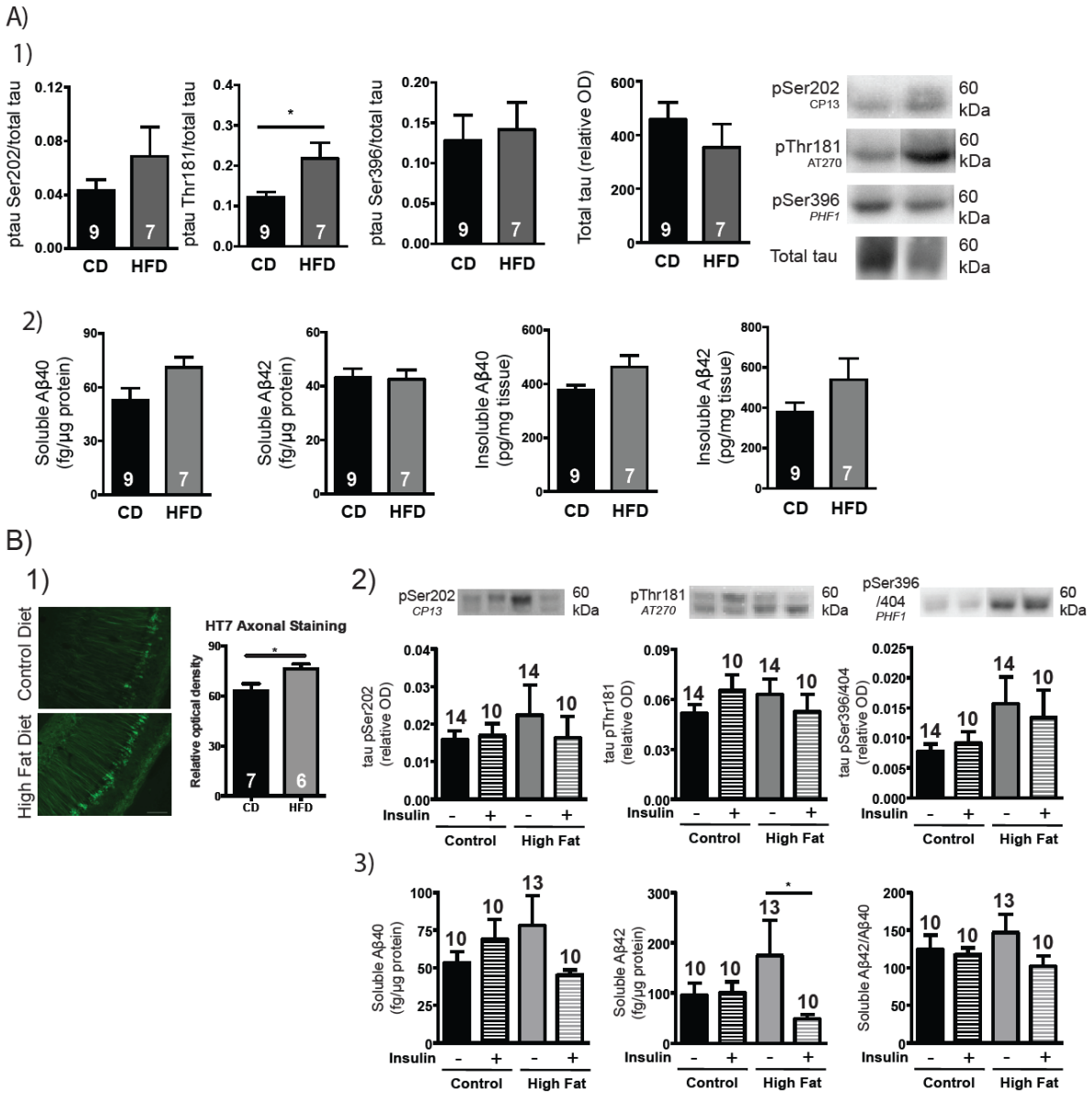
SUPPLEMENTARY DATA

Supplementary Figure 2. Effect of high-fat diet and insulin on tau and amyloid protein levels of 3xTg-AD mice A) High-fat diet increased tau phosphorylation but did not modulate amyloid pathology in 10-month-old 3xTg-AD mice.1) Cortex soluble tau pathology was measured by Western blot and 2) amyloid pathology was measured by ELISA after 4 months of high-fat diet. B) Insulin reduces hippocampal soluble Aβ42 in the high-fat fed 3xTg-AD mice. 1) Hippocampus tau immunofluorescence of 15-month-old 3xTg-AD mice fed the high-fat diet for 9 months. 2) Hippocampus soluble tau pathology measured by Western blot and 3) amyloid pathology measured by ELISA, 5 min. after IV insulin injection (3.8 U/kg).



Data are presented as mean ± SEM.
Data for immunofluorescence and ELISA experiment are compared using Student t-test and other data are compared using one-way ANOVA and Tukey's post-hoc analysis.
Data are compared using Unpaired Student t-test.
*$p < 0.05$.
CD = control diet; HFD = high-fat diet.

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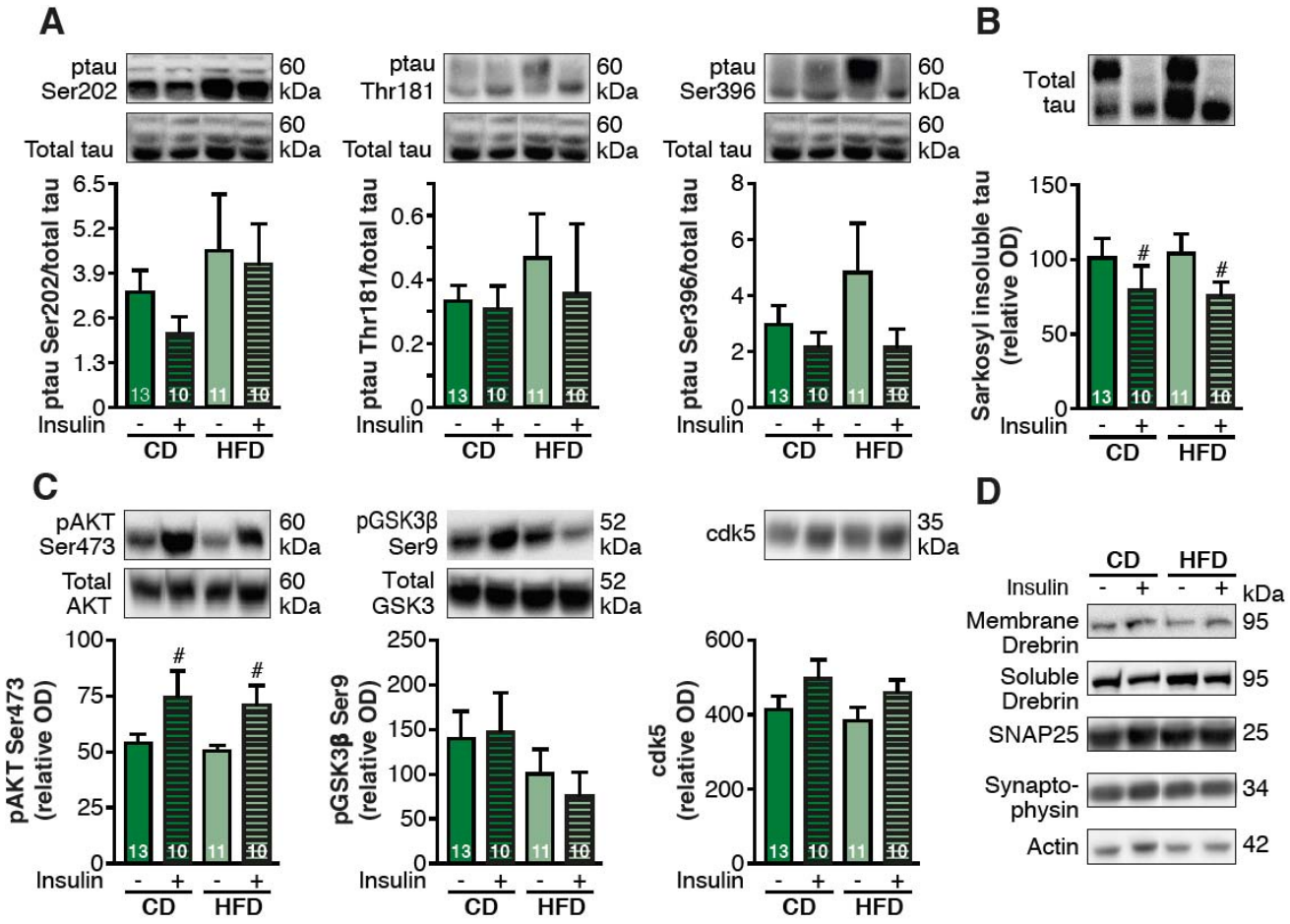
Supplementary Figure 3. Insulin has limited impact on cortical tau and synaptic pathology. Parieto-temporal cortex tau pathology of 3xTg-AD mice fed the control (CD) or the high fat diet (HFD) for 9 months 5 minutes after single insulin (3.8 U/Kg) or saline injection. A) Soluble ptau, B) sarkosyl-insoluble tau pathology and C) kinases concentration. D) Synaptic proteins in 15-month-old mice cortex.



Data are presented as mean ± SEM. n = 10 to 13 for biochemical analysis. To compare all groups separately, data are compared using one-way ANOVA followed by a Tukey's post-hoc analysis when variances were equal variances. *$p < 0.05$.
A Dunnett's post-hoc analysis was performed to compare groups when variances were not equal.
*$p < 0.05$, **$p<0.01$.
To verify the effect of insulin and dietary treatment a two-way ANOVA was performed. #$p < 0.05$.

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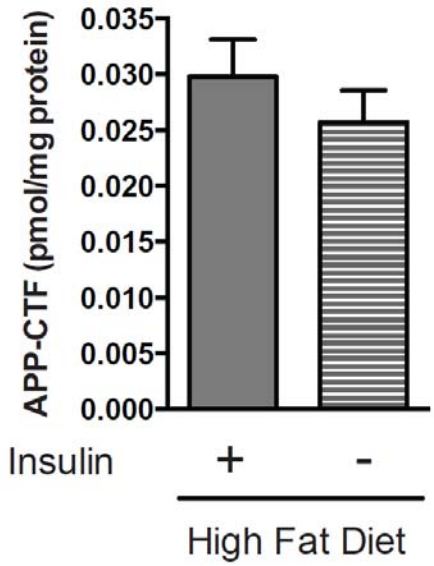
Supplementary Figure 4. Insulin had no effect on cortical APP-CTF concentration of 15-month-old 3xTg-AD mice fed the high-fat diet.
APP-CTF concentration was measured using an ELISA.



Data are presented as mean ± SEM.
Data are compared using Unpaired Student t-test.
no difference was found between groups.

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SUPPLEMENTARY DATA

Supplementary Table 1. Impact of high-fat diet ingestion and insulin injection on cortical synaptic proteins concentrations.

Protein	Control Diet						High Fat Diet						p value
	Saline (n=13)			Insulin (n=10)			Saline (n = 11)			Insulin (n=10)			
	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	
Soluble drebrin	104	±	41	100	±	67	78	±	25	92	±	38	ns
Membrane drebrin	12	±	5	11	±	6	11	±	5	12	±	6	ns
Synaptophysin	918	±	244	864	±	248	808	±	258	982	±	339	ns
SNAP25	653	±	127	634	±	110	701	±	159	714	±	192	ns

To compare all groups separately, data were compared using one-way ANOVA followed by a Tukey's post-hoc analysis. No statistical difference was found between groups.

Supplementary Table 2. Effect of insulin injection and high-fat diet ingestion on cortical concentrations of Aβ clearance related proteins.

Protein	Control Diet						High Fat Diet						p value
	Saline (n=13)			Insulin (n=10)			Saline (n = 11)			Insulin (n=10)			
	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	
Soluble IDE	184	±	49	190	±	61	168	±	33	169	±	45	ns
Membrane IDE	85	±	63	112	±	68	83	±	61	110	±	67	ns
RAGE	598	±	148	613	±	129	548	±	155	603	±	163	ns
LRP1	314	±	79	309	±	94,1	284	±	72,3	296	±	73	ns

To compare all groups separately, data were compared using one-way ANOVA followed by a Tukey's post-hoc analysis. No statistical difference was found between groups.

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Supplementary Table 3. List of antibodies used for Western blot, immunofluorescence and immunohistochemistry.

Antibody	Clone	Specificity	Host	Source
Aβ	6C3	monomeric Aβ40 and Aβ42	Mouse	EMD Millipore (Billerica, MA, USA)
ADAM10	polyclonal	c-term ADAM10, a.a. 732-748	Rabbit	EMD Millipore (Billerica, MA, USA)
AKT	polyclonal	AKT a.a. 345-480	Rabbit	Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA)
AKT (phospho)	polyclonal	AKT, phosphorylated at Ser-473	Rabbit	Cell Signaling Technology (Danvers, MA, USA)
APP/Aβ	6E10	APP a.a. 1-16	Mouse	Covance, Inc. (Princeton, NJ, USA)
BACE		Synthetic peptide	Mouse	EMD Millipore (Billerica, MA, USA)
Beclin	polyclonal		Rabbit	Anaspec (Fremont, CA, USA)
Cleaved-Caspase 3 (Asp 175)	polyclonal	Cleaved-Caspase 3 Asp 175	Rabbit	Cell Signaling Technology (Danvers, MA, USA)
cdk5	polyclonal	c-term cdk5	Rabbit	Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA)
CO1	ID6	Mitochondrial COX1	Mouse	Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA)
Drebrin	Mx823	c-term peptide (a.a.632-649)	Mouse	Progen Biotechnik GmbH (Heidelberg, Germany)
GFAP	GA-5	GFAP	Mouse	Sigma-Aldrich (St.Louis, MO, USA)
GSK3β		Rat GSK-3β aa. 1-160	Mouse	BD Biosciences (Mississauga, ON, Canada)
GSK3β (phospho)	polyclonal	GSK3β, phosphorylated at Ser-9	Rabbit	Cell Signaling Technology (Danvers, MA, USA)
IDE	polyclonal	IDE, 93-273	Rabbit	Abcam (Toronto, ON, Canada)
Insulin	polyclonal	Insulin	Rabbit	Cell Signaling Technology (Danvers, MA, USA)
LC3	EP15284	N-term, MAP1LC3A	Rabbit	Abcam (Toronto, ON, Canada)
LRP1	EPR3724	Synthetic peptide	Rabbit	Abcam (Toronto, ON, Canada)
PSD95	K28/43	PSD95	Mouse	Antibodies Incorporated (Davis, CA, USA)
RAGE	697023	RAGE, gly 23 Leu 342	Rat	R&D system (Minneapolis, MN, USA)
SNAP25	SM1 81	Whole Snap25	Mouse	Covance, Inc. (Princeton, NJ, USA)
Synaptophysin		Synaptophysin	Mouse	EMD Millipore (Billerica, MA, USA)
Tau	polyclonal	c-term Tau	Rabbit	Dako (Burlington, ON, Canada)
Tau (human)	Tau13	Total human tau	Mouse	Covance, Inc. (Princeton, NJ, USA)
Tau (paired helical filament)	HT7	PHF Tau, a.a. 159-163	Mouse	Pierce Endogen Inc. (Rockford, IL, USA)
Tau (phospho)	CP13	Tau, phosphorylated at Ser-205	Mouse	Generous gift from Peter Davies
Tau (phospho)	PHF1	Tau, phosphorylated at Ser-396 and Ser-404	Mouse	Generous gift from Peter Davies
Tau (phospho)	AT 270	Tau, phosphorylated at Thr-181	Mouse	Pierce Endogen Inc. (Rockford, IL, USA)
X11α	polyclonal	X11, a.a. 1-220	Rabbit	Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA)

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Protein extraction

The detailed procedure for protein extraction is described elsewhere (Lebbadi et al., 2011). Briefly, frozen parieto-temporal cortices were homogenized in tris-buffered saline (TBS) containing proteases and phosphatases inhibitors. Samples were centrifuged at 100,000 g for 20 minutes at 4°C to produce the soluble fraction : TBS-soluble intracellular and extracellular fraction. To produce the detergent-soluble fraction (membrane fraction), TBS-insoluble pellets were homogenized in RIPA buffer containing the same protease and phosphatase inhibitors. Part of the RIPA homogenate was used for sarkosyl extraction (see below) and the remaining homogenate was centrifuged at 100,000 g for 20 minutes at 4°C. The detergent-insoluble pellets were homogenized in 99% formic acid and centrifuged at 20,000 g for 20 minutes. The resulting supernatant was evaporated at room temperature for 48 to 72 hours. Part of the resulting pellet was resuspended in guanidine 50 mM for ELISA or Laemmli buffer for Western immunoblotting.

The isolation of the sarkosyl-insoluble fraction was based on a modified version (Julien et al., 2012) of the original protocol of Greenberg and Davies (Greenberg and Davies, 1990), 100 µl of the RIPA homogenate were centrifuged at 20,000 g for 20 minutes. Sarkosyl (1% v/v) was added to the supernatant, samples were put on rotating agitation at 37°C for 2 hours and were centrifuged for 1 hour at 100,000 g at 4°C. The supernatant was removed and the pellet was washed with 1% sarkosyl solution. Finally, the pellet was resuspended in Laemmli buffer for Western immunoblotting. Muscle and liver tissues were powderized and directly homogenized in RIPA buffer containing protease and phosphatase inhibitors. Homogenates were put on rotating agitation for 1 hour at 4°C and then centrifuged at 20,000 g for 20 minutes at 4°C. Laemmli buffer was added to the resulting supernatant and samples were denaturated at 95°C for 5 min for Western blot experiments.

APP-CTF quantification

Cortex APP-CTF were measured using a human β-CTF ELISA (Affinity Diagnostics Corp., Toronto, ON, Canada) according to manufacturer instructions and plates were read at 450 nm using a SynergyTM HT multi-detection microplate reader (Biotek, Winooski, VT).

Histology

Epididymal fat and pancreas were dissected, weighed and immediately post-fixed in paraformaldehyde 4% pH 7.4 for 48 hours. 10 µm sections were first hydrated in ethanol and stained in successive baths of hematoxylin (10 minutes), water (3 minutes) 70% alcohol and 1% HCl solution and washed with running water. Lithium carbonate dips, running water and eosin staining (30 seconds) were then performed prior to alcohol dehydration and coverslipping. Pancreatic islets area was measured under bright-field illumination using Stereo Investigator software (MicroBrightfield, Colchester, VT) integrated with an E800 Nikon microscope (Nikon Canada Inc., Mississauga, ON, Canada). Approximately 20 islets per animal were quantified with Neurolucida modelling software (MicroBrightfield).

Evaluation of locomotor activity and anxiety-like behavior.

Locomotor activity was evaluated using an open field system (San Diego Instruments, CA) consisting of 10 Plexiglas chambers (40 cm X 40 cm) during a one-hour session. Horizontal voluntary fine and ambulatory movements were detected using a Photobeam Activity System. Anxiety-like behaviour was evaluated using the dark-light emergence test (Latapy et al., 2012) performed in a box divided in two compartments (16 cm X 17 cm each): a dark one and a clear one with an opening between them (4 cm X 4 cm) to allow the mice to pass from one compartment to the other. The mice are placed in the center of the dark compartment. Time spent in each compartment was observed during a five minutes session.

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Immunofluorescence

For cerebral immunofluorescence experiments, 25-µm-thick slices were used and stained with 6E10 antibody to label amyloid plaques. Plaque size were measured in the hippocampus at bregma -2.7 mm using the Stereo Investigator software (MicroBrightfield) integrated with an E800 Nikon microscope (Nikon Canada Inc.). For pancreatic immunofluorescence, paraffin-embedded 10-µm pancreas sections from mice perfused with paraformaldehyde (4%, pH 7.4) were used. Detection of amyloid pathology of the pancreatic tissue was done using 6E10 and 6C3 antibodies. To be able to quantified number of 6E10 positive β-cells, a double staining was performed using an insulin antibody. Number of apoptotic β-cells in pancreatic islets was assessed with an antibody against cleaved caspase 3. Anti-mouse or anti-rabbit secondary antibodies conjugated with either Alexa Fluor 488 or 568 were used. Immunofluorescence was examined using an epifluorescence microscope (Olympus Provis AX70, Melville, NY) and photographs were taken using a Spot digital camera (Diagnostic Instruments, Sterling Heights, MI). All images were prepared for illustration in Adobe Photoshop 7.0.

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