SUPPLEMENTARY DATA

Dietary triglycerides act on mesolimbic structures to regulate the rewarding and motivational aspects of feeding.

AUTHORS AND AFFILIATIONS

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AUTHORS AND AFFILIATIONS

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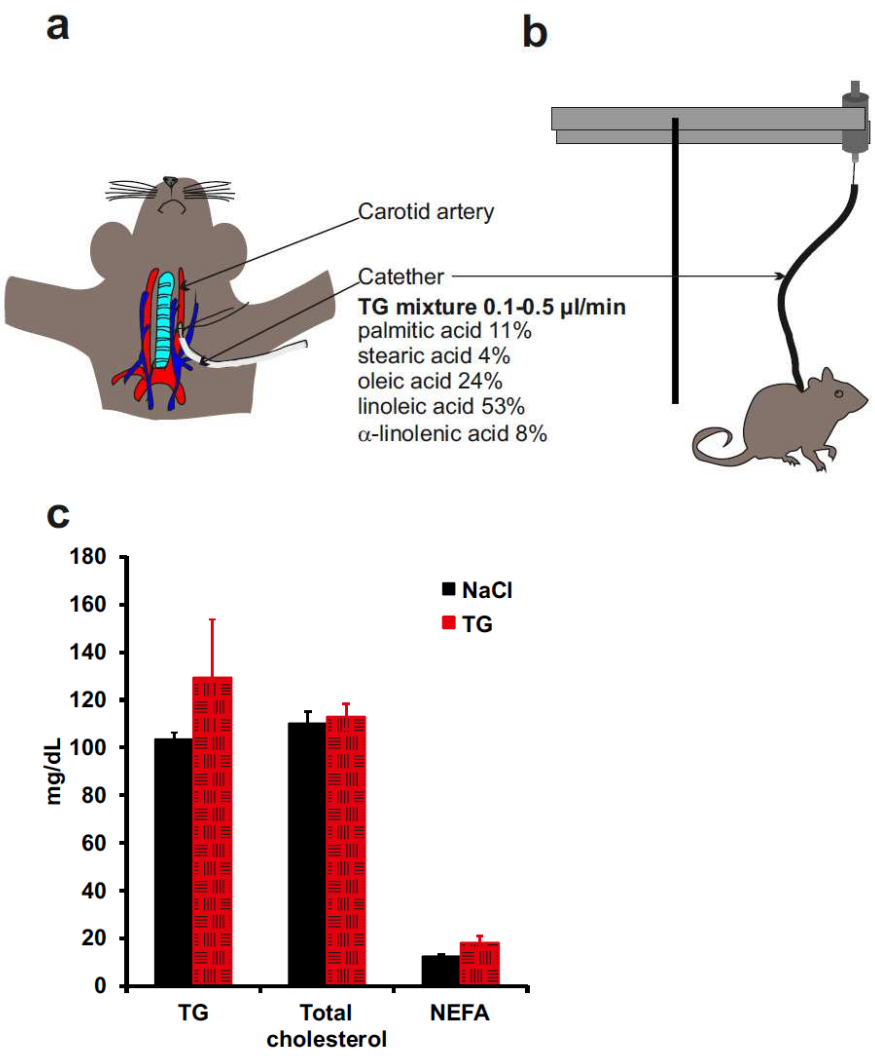
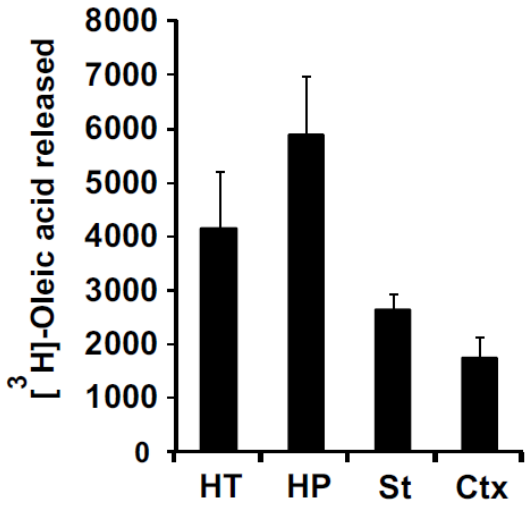
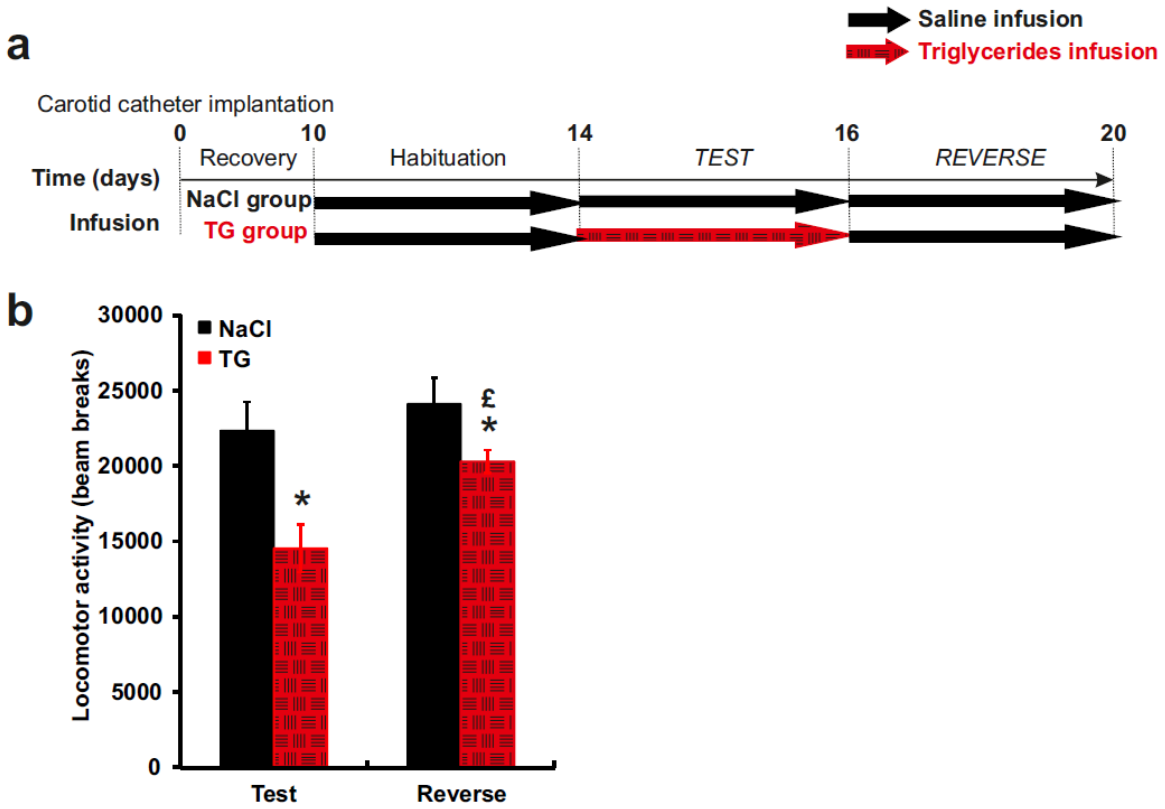
SUPPLEMENTARY RESULTS



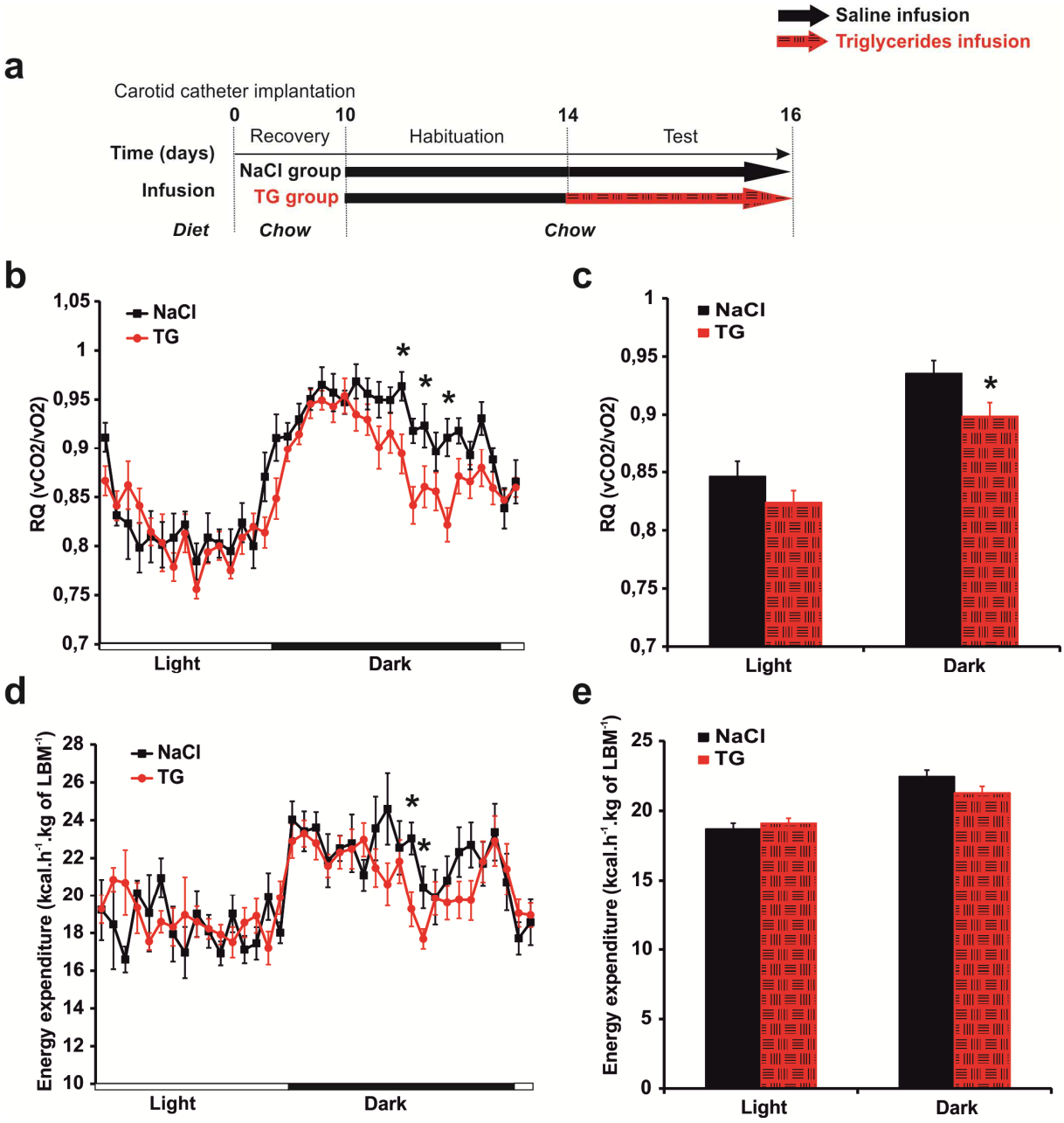
Supplementary figure 1. Establishment of a *in vivo* model for brain-specific triglycerides perfusion towards the brain. Surgical insertion of a catheter in the left carotid artery towards the brain (**a**). Experimental setting for triglycerides perfusion in freely moving animals (**b**). Plasmatic triglycerides (TG), total cholesterol and non-esterified fatty acids (NEFA) measured in mice receiving a 24-hrs NaCl (black bars) or TG (hatched red bars) solution into the carotid toward the brain (**c**). Displayed values are means ± SEM. (n=11).



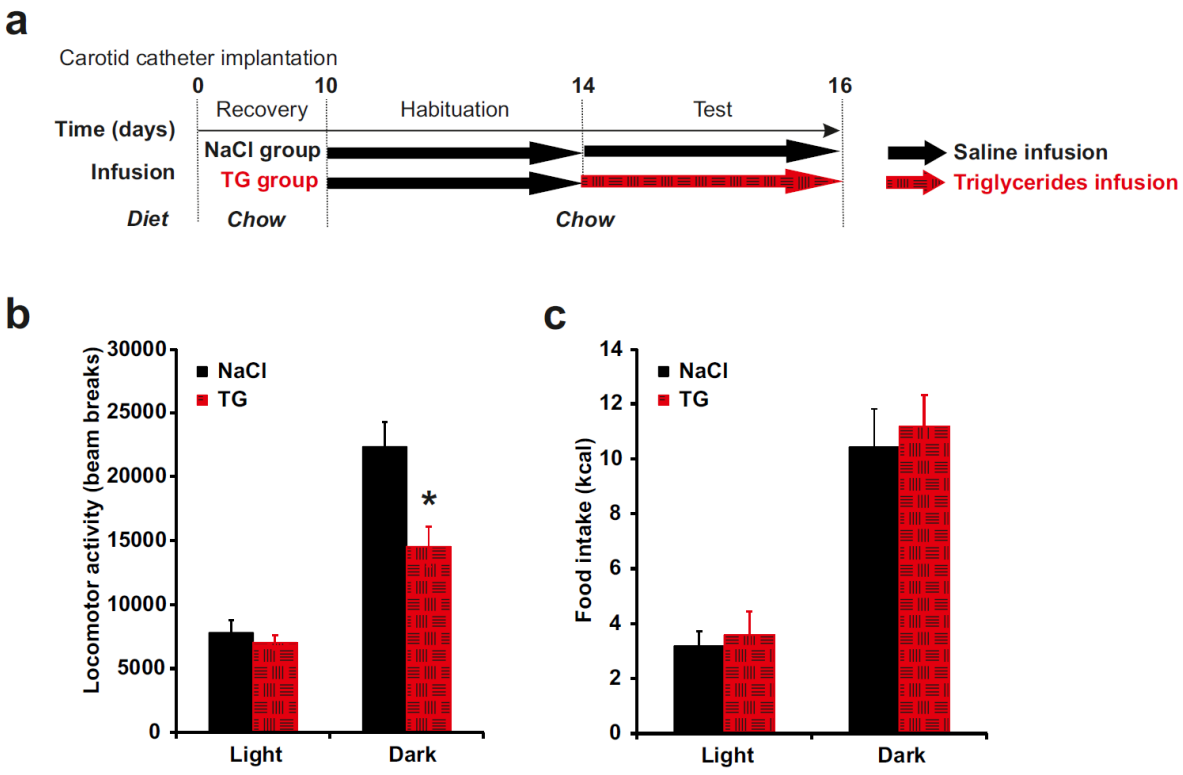
Supplementary figure 2. Nutritional triglycerides access the brain and are locally hydrolyzed. Distribution of [^{3}H]-Oleic acid radioactivity (cpm/g of tissue) after an intracarotid bolus of [9,10-^{3}H (N)]-Triolein (HT: Hypothalamus; HP: Hippocampus; St: Striatum; Ctx: Cortex). Displayed values are means ± SEM. (n=5-12).



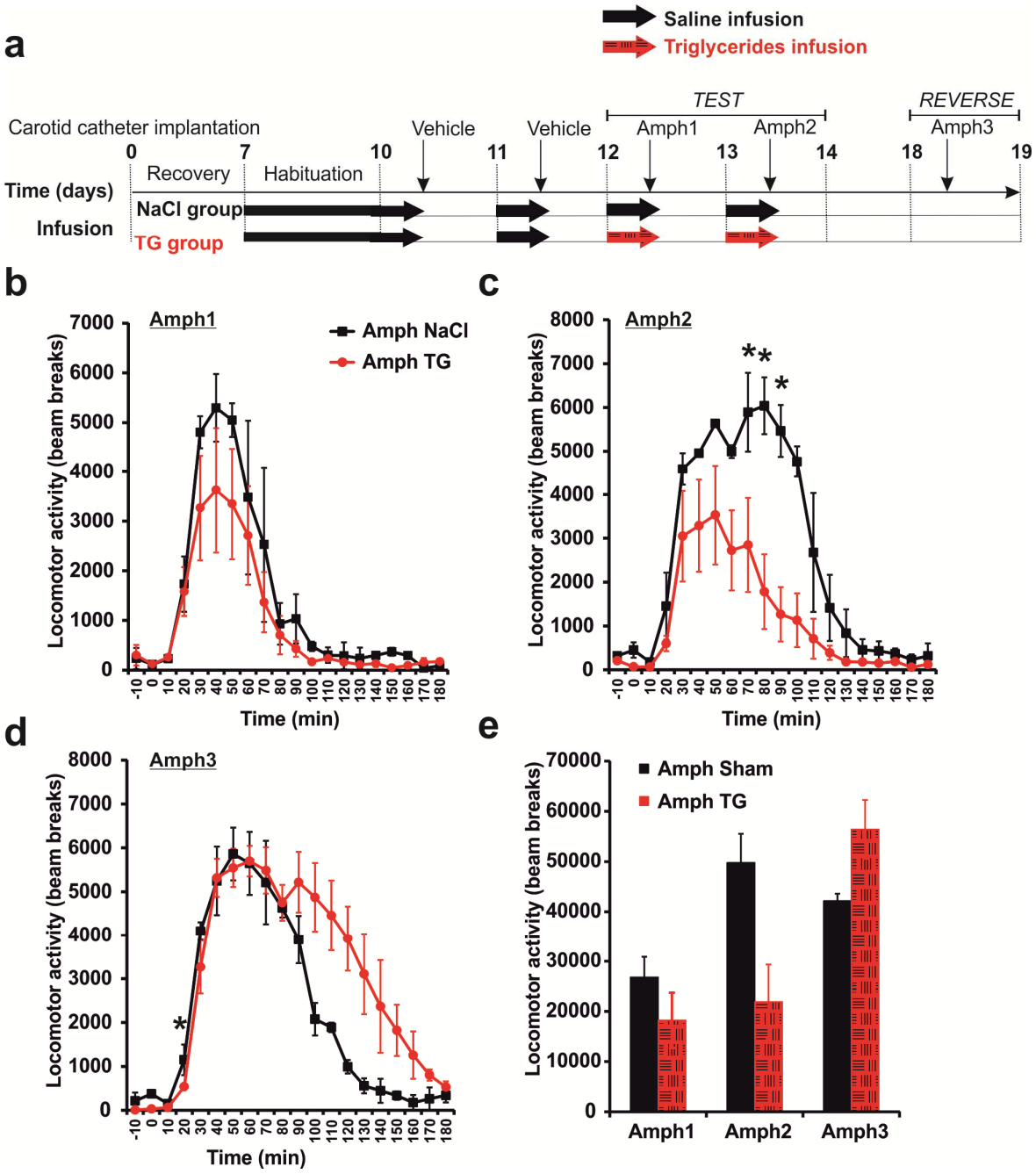
Supplementary figure 3. The action of central triglycerides delivery on spontaneous locomotor activity is reversible. Experimental procedure (**a**). Cumulative locomotor activity was recorded on animal group receiving NaCl (black bars) or TG (hatched red bars) infusion (Test phase) and then replaced on saline infusion for 4 days (Reverse phase) (**b**). Data present the mean of days 14+15 (Test phase) and 18+19 (Reverse phase). Displayed values are means ± SEM. (n=4-6). *****p<0,05 NaCl vs TG; **£**p<0,05 TG Test vs TG Reverse.



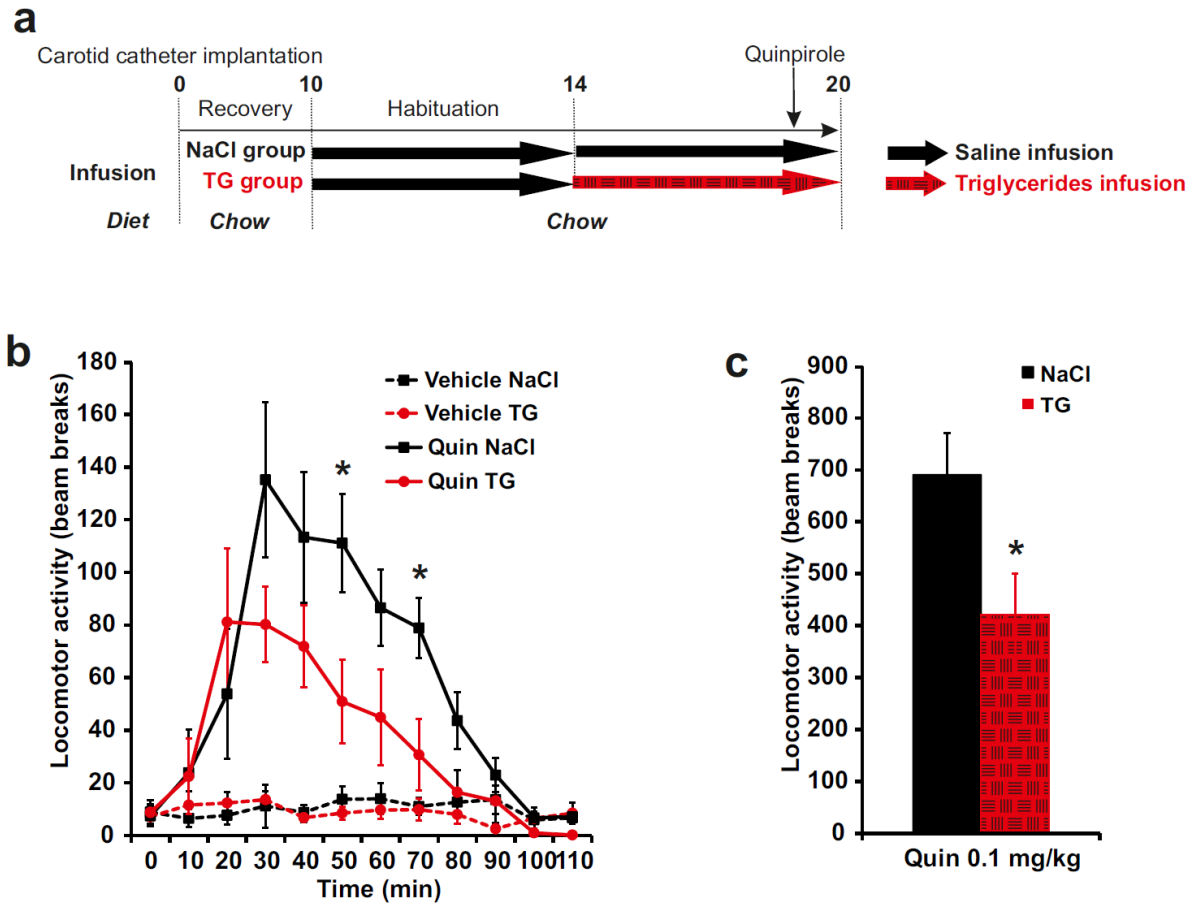
Supplementary figure 4. Central triglycerides delivery affects respiratory quotient and energy expenditure. Experimental procedure (**a**). Daily variation of respiratory quotient (**b**) and energy expenditure (**d**), medium respiratory quotient (**c**) and energy expenditure (**e**) during light and dark periods in control mice (black squares and bars) and triglycerides (TG) infused mice (red circles and hatched red bars). Data present the mean of days 14+15. Displayed values are means ± SEM. (n=5-6). *p<0,05 NaCl vs TG



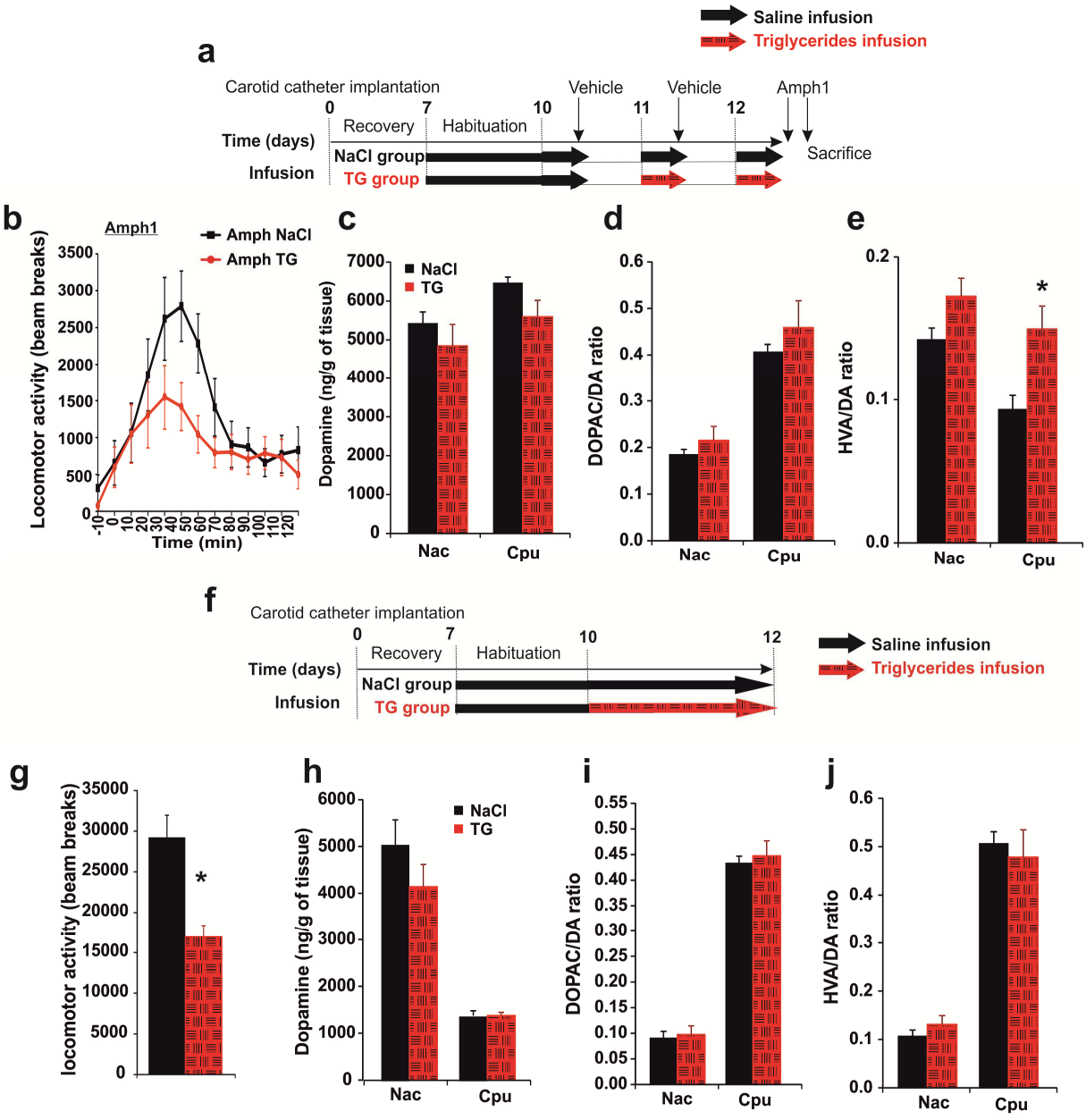
Supplementary figure 5. Central triglycerides delivery does not affect regular diet intake. Experimental procedure (**a**). Cumulative locomotor activity (**b**) and food intake (**c**) during light and dark periods in control mice (black bars) and triglycerides (TG) infused mice (hatched red bars). Data present the mean of days 14+15. Displayed values are means ± SEM. (n=5-6). *p<0,05 NaCl vs TG



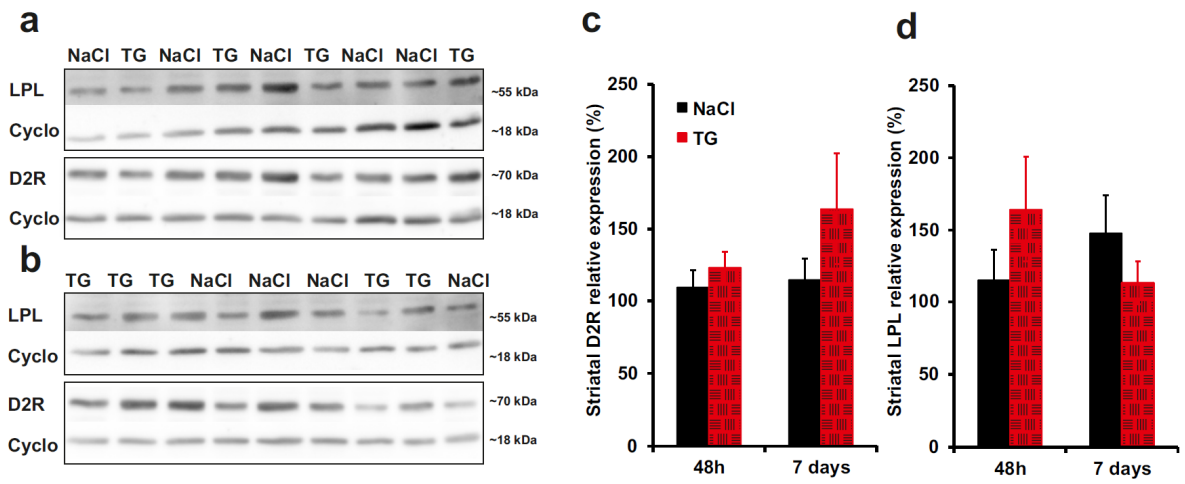
Supplementary figure 6. The action of central triglycerides delivery on amphetamine-induced locomotion is reversible. Experimental procedure (**a**). Locomotor activity was recorded during 3h after an acute intraperitoneal injection of 3 mg/kg D-amphetamine or vehicle (saline solution) in control mice (black square) and TG infused mice (red circles) (Test phase) (**b,c**) and then TG infusion is stopped for 4 days and both groups are injected with D-amphetamine again (Reverse phase) (**d**). Cumulative locomotor activity during the Test and Reverse phase in control mice (black bars) and TG infused mice (hatched red bars) (**e**). Mice were infused with NaCl or TG solution during 6h just before vehicle or D-amphetamine injection (Vehicle and Test phase). Displayed values are means ± SEM (n=2-5). *****p<0,05 NaCl vs TG. A repeated measure ANOVA with time and groups as factor shows a stronger lasting effect of amphetamine in Amph TG group (p=0,023) (**d**).



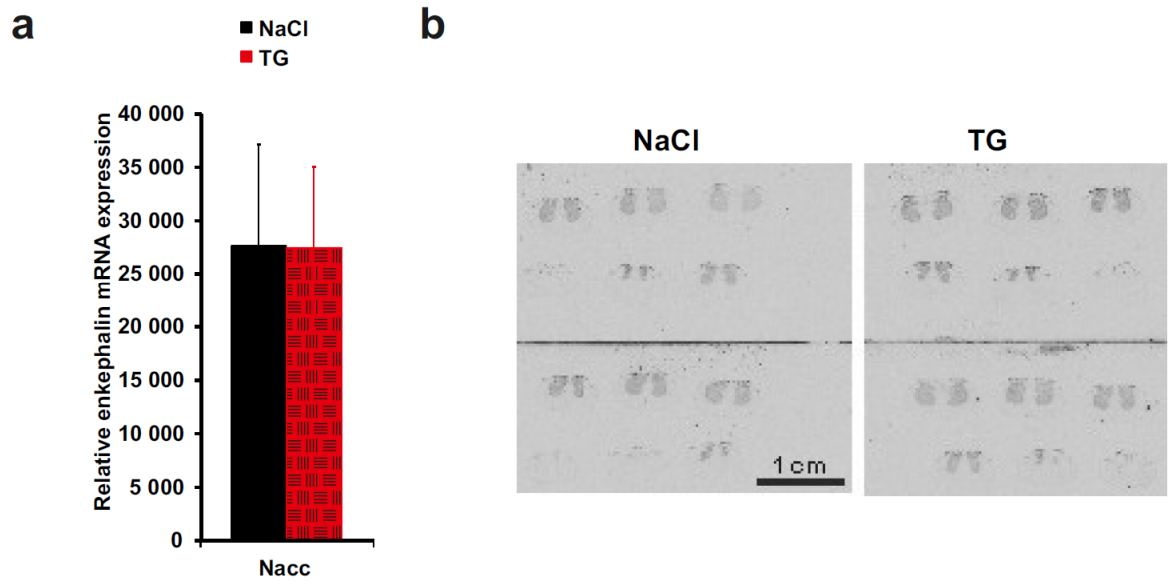
Supplementary figure 7. Central triglycerides delivery decreases Quinpirole-induced locomotion. Experimental procedure (**a**). Locomotor activity evolution (**b**) and cumulative locomotor activity (**c**) were recorded during 2h after an acute intraperitoneal injection of vehicle (saline solution) or Quinpirole (0.1 mg/kg) in mice infused with NaCl (black square and bars) or TG (red circles and hatched red bars) into the carotid toward the brain during 6 days. Displayed values are means ± SEM. (n=5-6). *p<0,05 NaCl vs TG.



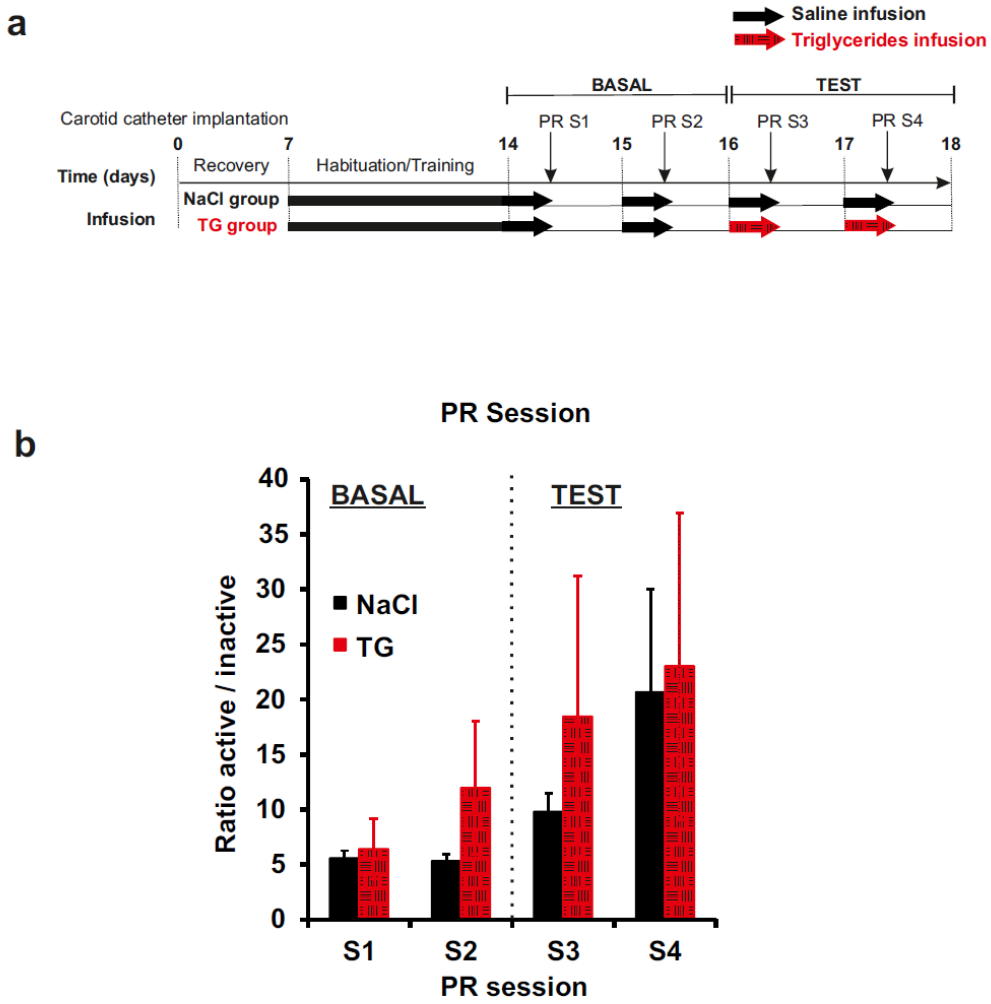
Supplementary figure 8. Central triglycerides delivery decreases spontaneous and amphetamine-induced locomotion with moderate impact on striatal dopamine or dopamine metabolite content. Experimental procedure (**a, f**). Cumulative locomotor activity was recorded during 2h after an acute intraperitoneal injection of vehicle (saline solution) or 3 mg/kg D-amphetamine (**b**) or over a 24-hrs period (**g**) in mice receiving a 6-hrs (**a, b**) or a 48-hrs (**f, g**) intra-carotid saline (black circles and bars) or TG infusion (red circles and hatched red bars). Dopamine (**c, h**) and dopamine metabolite (dihydroxyphenylacetic acid (DOPAC) and homovanillic (HVA)) were measured by High-performance liquid chromatography (HPLC) in Nucelus accumbens (Nac) and the Caudate putamen (Cpu) of mice sacrificed 2-hrs after amphetamine injection(**c-e**) or after a 48-hrs saline or TG infusion (**h-j**). The ratio of DOPAC/DA (**d, i**) and HVA/DA (**e, j**) are indicative of dopamine turn-over. Displayed values are means ± SEM. (n=5-6 per group). ***p<0,05 NaCl vs TG.



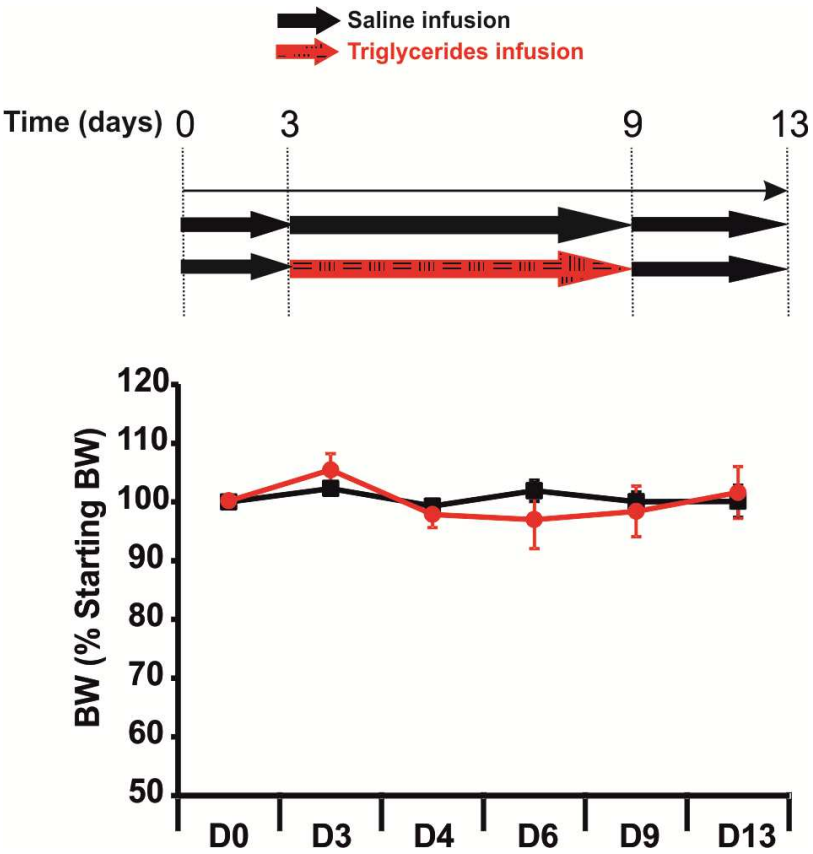
Supplementary figure 9. Central triglycerides delivery does not alter striatal lipoprotein lipase or dopaminergic receptor 2 expression. Dopaminergic receptor 2 (D2R) and lipoprotein lipase (LPL) protein abundance was measured in total striatal extract by western blot in mice receiving NaCl (black bars) or TG (hatched red bars) solution into the carotid toward the brain for 48h (a) or 7 days (b). D2R (c) and LPL (d) expression in % of cyclophilin expression. Displayed values are means ± SEM. (n=5-6).



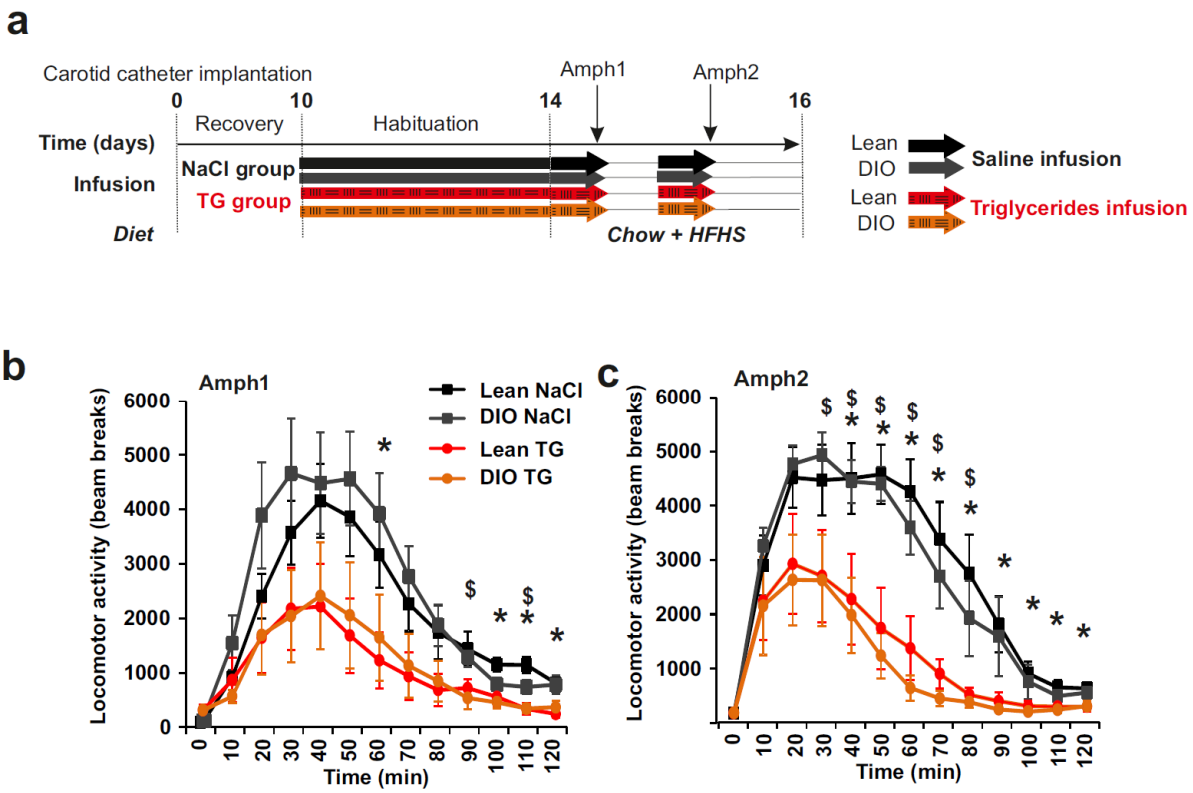
Supplementary figure 10. Central triglycerides delivery does not alter striatal enkephalin mRNA expression. Relative enkephalin expression in the Nucleus Accumbens (Nacc) was measured by in situ hybridization in mice receiving NaCl (black bars) or TG (hatched red bars) solution into the carotid toward the brain for 24h (**a**). Representative photo-autoradiogram of brain section hybridized with encephalin anti-sense probe (**b**). Displayed values are means ± SEM. (n=6).



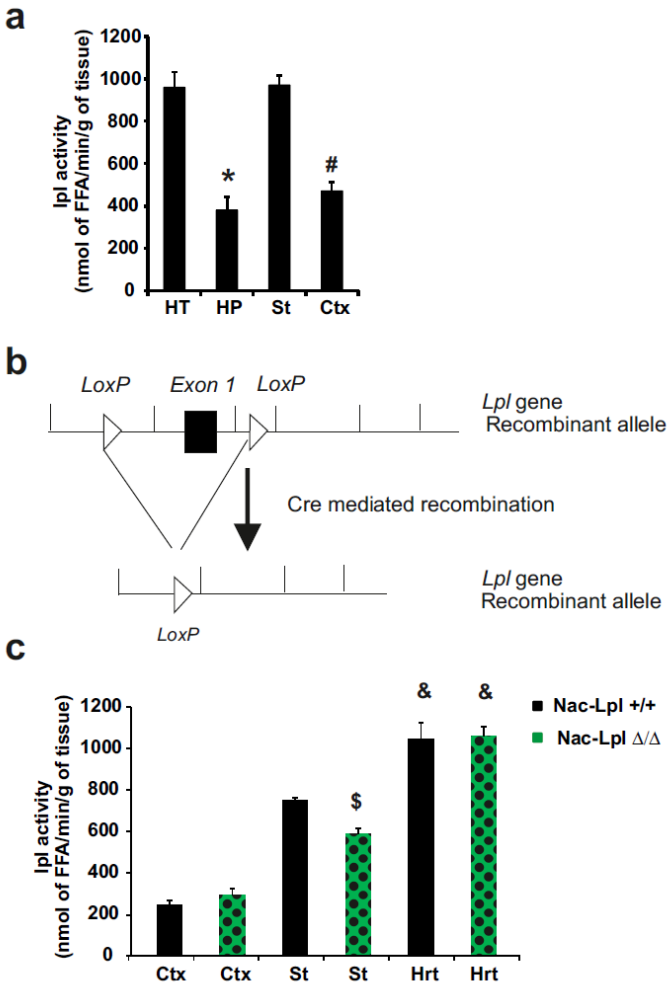
Supplementary figure 11. Central triglycerides delivery specifically decreases motivational aspect of reward seeking. Experimental setting **:** mice were infused with NaCl or TG solution during 6h just before session of PR; during basal conditions both group were infused with saline solution (**a**)**.** Ratio of active/inactive lever presses achieved were recorded during 4 sessions of PR in control mice (black bars) and TG infused mice (red circles and hatched red bars) (**b**). Displayed values are means ± SEM. (n=5).



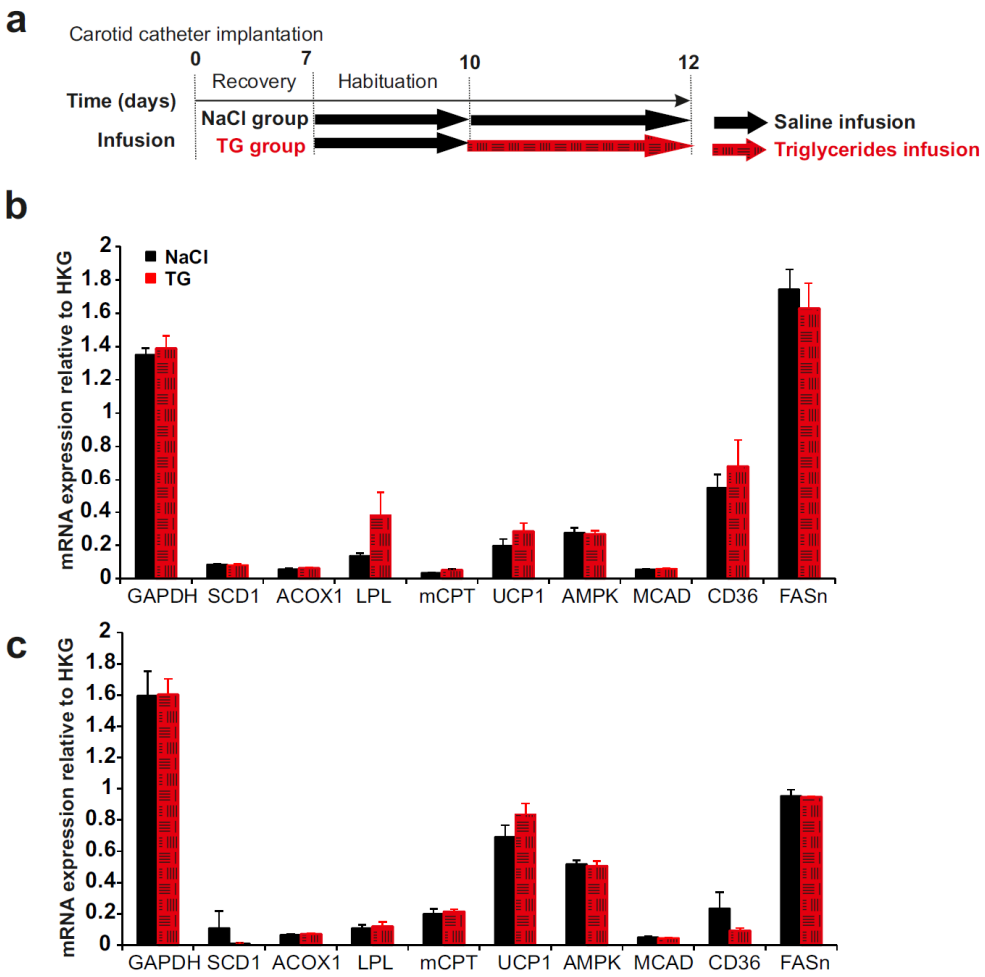
Supplementary figure 12. Central triglycerides delivery does not affect body weight. Body weight (% of initial) was measured in mice receiving NaCl (black square and arrow) or TG (red circles and hatched red arrow) solution into the carotid toward the brain for 7 days. Displayed values are means ± SEM. (n=5-6).



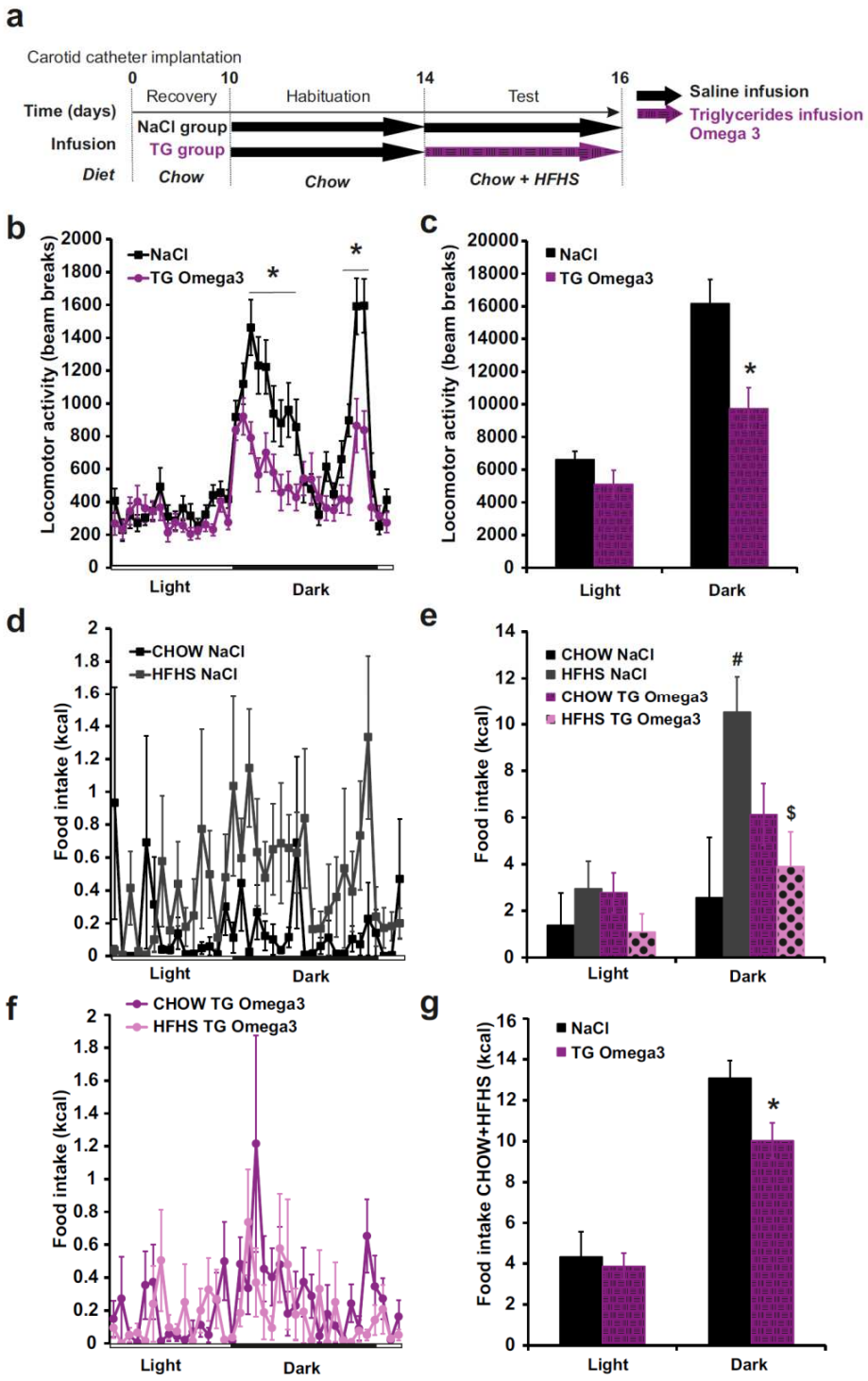
Supplementary figure 13. Central triglycerides delivery decreases amphetamine-induced locomotion in lean and obese mice. Experimental procedure **(a).** Cumulative locomotor was recorded during 2h after repeated intraperitoneal injection of 3 mg/kg D-amphetamine in lean mice infused with NaCl (black square) or TG (red circles) and in DIO mice infused with NaCl (grey squares) or TG (oranges circles). Mice were infused with NaCl or TG solution during 6h just before vehicle or D-amphetamine injection. Displayed values are means ± SEM. (n=6). ***p<0,05 NaCl vs TG in lean mice; **$***p<0,05 NaCl vs TG in DIO mice.



Supplementary figure 14. LPL activity in brain structures.. Lipoprotein lipase activity was measured in brain punches of control mice (**a**). Schematic of the *Lpl* gene locus in *Lpl* $^{lox/lox}$ mice genetically engineered to allow for selective CRE-mediated recombination of the first exon of the gene encoding the lipoprotein lipase (**b**). Lipoprotein lipase activity was measured in brain punches and heart of control mice (black squares and bars) and in mice with a NAc-specific Lpl knock down (green circles and green dotted bars) (**c**). (HT: Hypothalamus; HP: Hippocampus; St: Striatum; Ctx: Cortex; Hrt: Heart). Displayed values are means ± SE (n=4-8). *p<0,05 HP vs HT and St; #p<0,05 Ctx vs HT and St; $ p<0,05 NAc-*Lpl*$^{+/+}$ vs NAc-*Lpl*$^{\Delta/\Delta}$; & p<0,05 Hrt and St.



Supplementary figure 15. Central triglycerides delivery does not affect transcription of gene involved in lipid metabolism. Experimental procedure (**a**). mRNA level encoding gene involved in lipid metabolism were assessed using real-time PCR on striatal (**b**) or cortex (**c**) punches of mice perfused with saline (black bars) or triglycerides (TG, hatched red bars) in the carotid artery in the direction of the brain for 48 hours. Data present average value of mRNA relative to house keeping gene (HKG) for the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Stearoy-CoA desaturase (SCD1), Acyl-CoA oxidase 1 (ACOX1), Lipoprotein lipase (LPL), Carnitine palmitoyl transferase 1 (mCPT1), (Uncoupling protein 1) UCP1, Protein kinase AMP-activated (AMPK), Acyl CoA dehydrogenase (MCAD), Fatty transporter CD36 FAT (CD36), Fatty Acid Synthase (FASn). Displayed values are means ± SEM. (n=5-6).



Supplementary figure 16. Central triglycerides enriched in omega 3 delivery specifically decreases nocturnal locomotor activity and abolishes feeding preference for palatable food. Experimental procedure (**a**). Daily variation of locomotor activity (**b**) and cumulative locomotor activity during light and dark periods (**c**) in control mice (black squares and bars) and triglycerides (TG Omega3) infused mice (purple circles and hatched purple bars). Daily variation in chow (black squares) and HFHS (gray squares) diet intake in control mice (**d**). Daily variation in chow (purple circles) and HFHS (pink circles) diet intake in TG infused mice (**e**). Cumulative food intake during light and dark periods of chow diet (control mice: black bars; TG infused mice: hatched purple bars) and HFHS diet (control mice: grey bars; TG infused mice: dotted pink bars) (**f**). Cumulative total food intake (chow+HFHS diet) during light and dark periods in control mice (black bars) and TG infused mice (hatched purple bars) (**g**). Data present the mean of days 14+15. Displayed values are means ± SEM. (n=5-6). *p<0,05 NaCl vs TG Omega3; #p<0,05 CHOW NaCl vs HFHS NaCl; $p<0,05 HFHS NaCl vs HFHS TG Omega3.

SUPPLEMENTARY EXPERIMENTAL PROCEDURE

Animals and diets

Food and water were given *ad libitum* unless otherwise. Food (Safe, Augy, France) was either Chow diet (3200 kcal/kg protein 21.4%, fat 5.9%, carbohydrate 51.7%) or High Fat High Sucrose diet for two choices procedure (4362 kcal/kg protein 19.8%, fat 23.0%, carbohydrate 37.5%). Lipoprotein lipase LPL $^{lox/lox}$ mice [52] were obtained from the Jackson laboratory, strain B6.129S4-Lpl^{tm1Ijg}/J, n° 00650. Diet-induced obese animals were fed for 6 months with high fat diet, (5558 kcal/kg protein 23%, fat 58%, carbohydrate 25.5%, reference #D12331 Research Diet, New Brunswick, USA) containing. Two choices experiment with DIO and lean control were performed using chow (3200 kcal/kg protein 21.4%, fat 5.9%, carbohydrate 51.7%) and High fat High sucrose diet (4057 kcal/kg protein 20%, fat 60%, carbohydrate 20%, reference #D12492 Research Diet, New Brunswick, USA)

Infusion procedures

Intralipid® 20% (Fresenius Kabi, Sèvres, France) is a TG emulsion that contains 53% linoleic acid ; 24% oleic acid ; 11% palmitic acid ; 8% alpha-linoleic acid ; 4% stearic acid and Omegaven® (Fresenius Kabi, Sèvres, France) is a TG emulsion derived from fish oil that is selectively enriched in omega 3 that contains 1,25-2,82g of eicosapentaenoic acid (EPA) and 1,44-3,09g of docosahexaenoic acid (DHA) in 100ml. Mice infused with Intralipid® are named TG mice, mice infused with Omegaven® are named TG Omega3 and one still infused with saline are named NaCl mice. Mice are infused for 1 hour, 6 hours, 2 days or 7 days. Animals received

TG or NaCl infusion +heparin (20U/ml). The rate of infusion was 0.1-0.5 µl/min. Rate of central lipid perfusions were calculated so as to approximate physiological concentration of TG entering the brain. Mice have a central blood flow of about 0.7-1.7 ml/min/g tissue [64] and a blood TG concentration of ~0.8-0.9 mg/ml [64]. A mouse brain weighs ~0.5g which represents (0.7-1.7)*0.5*(0.8-0.9) =0.28-0.68 mg of TG entering the brain/min. This value compares to 0.1-0.5µl/min of 20% IL which is 0.02*0.1-0.5 mg of TG/min infused, representing an average increase 6-30% of the plasma TG through normal blood flow.

In vivo lipid delivery and measurement of locomotor activity, food intake respiratory quotient and energy expenditure. Metabolic and behavioral analysis were performed on freely moving animals perfused with lipid or saline trough a swiveling device specifically adapted to phenomaster system of the Functional & Physiological Exploration Platform (FPE) of the Unit "Biologie Fonctionnelle et Adaptative", Univ Paris Diderot, Sorbonne Paris Cité, BFA, UMR 8251 CNRS, F-75205 Paris, France as previously described [65]. Animals were individually housed in a cage with lights on from 0700 to 1900 hours and an ambient temperature of 22 ± 3°C. All experimental events were controlled and recorded every minute by computers during the same period in the light/dark cycle. Briefly, O2 consumption, CO2 production, energy expenditure, food intake and locomotor activity were recorded every 1, 10 or 40 min for each animal during the entire experiment. Food consumption was recorded as the instrument combined a set of highly sensitive feeding sensors for automated online measurement. Mice had free access to food and water ad libitum. To allow measurement of every ambulatory movement, each cage was embedded in a frame with an infrared light beam-based activity monitoring

system with online measurement at 100 HZ. Throughout the experiment, mice were monitored for body weight and composition at the entry and the exit of the experiment. Body mass composition (lean tissue mass, fat mass, free water and total water content) was analyzed using an Echo Medical systems' EchoMRI (Whole Body Composition Analyzers, EchoMRI, Houston, USA), according to manufacturer's instructions

Operant conditioning system

Experiment 5 (central triglycerides delivery): After catheter implantation, operant training was carried out over 4 consecutive days with overnight fixe ratio (FR) 1 and then 3 consecutive days with one 2-hr trial of FR1 per day. At the conclusion of the 7-day operant training regimen, animals were given 4 trials to lever press for sucrose under a progressive ratio (PR) 3 schedule of reinforcement (lever press requirement for each subsequent reinforcer increased by 3 with an initial requirement of 3 lever press r=3N+3; N=reinforcer number). During the training regimen and the first two days of PR3 schedule mice were infused with NaCl solution into the carotid toward the brain. For subsequent PR3 sessions animals were divided in two groups: one group was infused with saline and the other one was infused with triglycerides. Animals were infused each day during 6h before the PR3 sessions.

Experiment 8 (Nac-specific Lpl knock down): Operant training was carried out over 2 consecutive days with overnight fixe ratio (FR) 1 and then 3 consecutive days with one 2-hr trial of FR1 per day. At the conclusion of the 5-day operant training regimen, animals were given 4 trials to lever press for sucrose under a progressive ratio (PR) 3 schedule of reinforcement.

LPL activity assay

Heparin-releasable LPL activity was assayed in brain regions using a Roar LPL activity assay kit (RB-LPL, Roar Biomedical, Inc.). Briefly, tissues were lysed in 500 µL of assay buffer (150 mM NaCl, 10 mM Tris, 2 mM EDTA, pH 7.4) and incubated for 45 min at 37°C with an equivalent volume of heparin (100U/mL). After incubation, samples were centrifuged for 10 min at 3000 g, and 10 µl of the aqueous phase deposited on LPL substrate emulsion in 96-well black microplates (VWR International, # 25227-304) and incubated for 1h at 37°C. Finally, fluorescence was read using a fluorimeter (370 nm excitation / 450 nm emission) and compared to a standard curve 18 made using known concentrations of pre-hydrolyzed LPL substrate. LPL activity was expressed as nanomoles of free fatty acid produced per minute per gram of proteins or tissue.

Free fatty acids extraction and concentration

Lipids were extracted by a modification of the method of Bligh and Dyer [66]. The tissues were homogenized in 1.8 ml of chloroform/methanol/HCl (50:100:1, v/v/v) and left at room temperature for 30 min. One-half milliliter of H_20 was then added and, after a brief homogenization, the monophase was split by the addition of 0.6 ml of 2 M KCl and 0.6 ml of chloroform. After a vigorous mixing, phases were separated by a 10-min centrifugation at 1000 × g and the aqueous phase was removed. The chloroform extract was dried with a speed vacuum concentrator and was then resuspended in 50 µl of methanol/chloroform (95:5, v/v). 500µl of Dole's lipid extraction media was added and then 200µ of n-heptane plus 300µl H_2O. After 30s of gentle mixing, and 5 min incubation at 22-23°C, samples free fatty acids were

extracted by a 10-min centrifugation at 1000 x g. Supernatants containing free fatty acids were collected for radioactivity counting procedure. Free fatty acids concentrations were measured using an enzymatic assay (NEFA-C test; Wako Chemicals).

Triglycerides, total cholesterol and non-esterified fatty acids concentrations

Plasma total cholesterol (Thermo Fisher), triglycerides (Thermo Fisher) and free fatty acids (Wako Chemicals) were measured by enzymatic assay kits. All assays were performed according to the manufacturers' instructions.

Brain specific Triolein hydrolysis

100-µl bolus of Intralipid® solution containing 40µl of ^{3}H triolein (0.5mCi/ml, Perkin Elmer) was injected rapidly into the carotid artery towards the brain (Cf. catheter implantation paragraph). Mice were decapitated 2.5 min after injection and brains removed quickly and dissected. Radioactivity of the aqueous free fatty acid phase containing ^{3}H Oleic acid were determined by a dual-label counting procedure in a Packard Tri-Carb 2100 TR liquid scintillation analyzer (Cf. Lipid extraction paragraph). These techniques of injecting a bolus dose of radiolabelled substance into the carotid artery does not permit mixing with the blood before the measurements are taken. The bolus dose fills the artery and the animals are killed after one passage through the brain. This technique is therefore not influenced by blood oleic acid and triolein concentration.

Fluorescent in Situ Hybridization

Antisense riboprobes against mouse LPL 3'UTR (*NM_008509*) were generated using an *in vitro* transcription kit with digoxigenin-labeled uridine triphosphate (Roche Applied Science, Indianapolis, Indiana). Fresh frozen mouse brains were cryosectioned at 14µm and mounted onto slides. Sections were fixed in ice-cold 4% paraformaldehyde for 20min, dehydrated in a series of ethanol gradients and allowed to dry. Sections were then rehydrated and acetylated for 5min. After drying sections,

the tissue was incubated with LPL riboprobes at 60°C overnight in hybridization buffer (50% formamide, 53 SSC, 53 Denhardt's, 250 mg/ml yeast RNA, and 200–300 ng/ml RNA probe). Sections were washed in 1x PBS and blocked with 5% normal sheep serum and 1% blocking reagent (Roche). LPL riboprobes were detected incubating sections with a 1:200 dilution of peroxidase-conjugated sheep anti-digoxigenin antibody (Roche). Fluorescent signal was achieved using TSA-Cy3 (Perkin-Elmer, Wellesley, Massachusetts).

Measure of brain catecholamine content

Catecholamine analyses were performed using the Bioprofiler platform of the Unit "Biologie Fonctionnelle et Adaptative", Univ Paris Diderot, Sorbonne Paris Cité, BFA, UMR 8251 CNRS, F-75205 Paris, France.The weighed tissues were suspended in 400 µL of ice-cold solution 0.1M perchloric acid containing 0.4% EDTA, and homogenized for 2 mn using a tissue lyser II (Qiagen), then the homogenates were centrifuged at 3000 × t/mn for 30 min at 4 °C, the supernatant was centrifuged at 16,000 g for 2 mn and analysis for monoamine content on the same day. The monoamine and metabolite concentration in the brain were analyzed by means of electrochemical detection (Waters 2465, Shimadzu) at a potential of 750mV following reverse-phase liquid chromatography (HPLC), with a 20µl sample loop leading to a column (Kromasil, 150*4.6mm, 5µ C-18 packing). The mobile phase consisted of an acetate buffer containing 100µM EDTA, 1mM octanesulfonic acid, and 15% v/v methanol (pH 3.1) and which was delivered at a constant flow rate of 1ml/min. This system allowed detection of monoaminergic compounds including dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic (HVA), with a limit detectability of 0.5pg/injected volume.

Enkephalin in situ hybridization

After infusion experiment (24h of Intralipid® or saline infusion into the carotid toward the brain), brain are gently removed and frozen. mRNA levels of ENK were measured using radiolabeled ISH. Antisense RNA probes were labeled with 33P-UTP (PerlinElmer, Boston, USA). 20 µm coronal sections on gelatine coated glasses (see c-fos procedure) were consecutively processed as follows: 10 min in 4% paraformaldehyde, and 10 min in acetylation solution, with a 10-min wash in PBS between each step. After the wash, the sections dehydrated and hybridized with a 33P-labeled probe (103 cpm/mL) at 72 °C overnight. Following hybridization, the sections were washed in 5× and subsequently 0.2X sodium citrate (SSC) for 2 hours. Sections were then run through ethanol with increasing percentages (30, 50, 70, 95 and 100%) diluted with ammoniumacetate. Sections were finally air-dried, and exposed to a Kodak BioMax MR film (Sigma-Aldrich, Steinheim, Germany) for 4 days at room temperature. All images (1600 dpi) were saved as Tiff files and analyzed using the public domain Java image processing program ImageJ (Rasband, WS, US National Institutes of Health, Bethesda, MD, USA, http://rsbweb.nih.gov/ij/)

Quinpirole injection

During 7 days triglycerides infusion towards the brain (as described in infusion procedures paragraph), the 3Th day mice were given i.p injection of vehicle solution and the 6Th day 0.1 mg/kg i.p. injection of Quinpirole (1061, Tocris) at the onset of the dark period of the light/dark cycle. Locomotor activity for each animal was measured throughout the test.

Western Blot analysis

After 24h or 7days of triglycerides infusion toward the brain (as described in infusion procedure paragraph) mice were killed and brain dissected. Tissue was resuspended in 300µl cell lysis reagent (C3228, Sigma) containing 1% protease inhibitor cocktail (P8340, Sigma). Tissue lysates were boiled for 10 min, centrifuged at 10.000 rpm for 10 min, then supernatants were loaded onto 15% Tris-HCl Polyacrylamide gel (161-1157, Bio-Rad). Protein was transferred to PVDF membranes, blocked for 1h at 23-25°C (5% non-fat dry milk and 0.1% Tween-20 in TBS, pH 7.4) and incubated in primary antibody overnight at 4°C. The following primary antibodies were diluted in block solution: anti-Dopamine D2 receptor (1/1000, AB5084P, Millipore), anti-Lipoprotein Lipase (1/200, sc-32885, Santa Cruz) and anti-Cyclophilin A (1/5000, 07-313, Millipore). Chemiluminescent ECL reagent was added after incubation with Anti-Rabbit IgG Peroxidase-conjugated for 1h at 23-25°C (1/10.000, A0545, Sigma). The membrane associated form of D2R (~70kDa) and LPL (~55kDa) were normalized to a protein loading control (Cyclophilin A ~18kDa) and quantified by densitometry using MultiGauge software.

Isolation of total RNA and quantitative RT-PCR in brain punches.

Total RNA were isolated from cortex and nucleus accumbens using RNeasy Lipid mini kit (Qiagen, Courtaboeuf, France). To remove residual DNA contamination, the RNA samples were treated with DNAseRNAse-free (Qiagen, Aubervilliers, France). Two µg of total RNA from each sample was reverse transcribed with 40 U of M-MLV Reverse Transcriptase (Invitrogen, life technologies, Logan, UT, USA) using random hexamer primers. Real time quantitative PCR amplification reaction were carried out in a LightCycler 1.5 detection system (Roche, Meylan, France) using the Light-

Cycler FastStart DNA Master plus SYBR Green I kit (Roche). The PCR conditions were: 95 ° C for 10 min, followed by 40 cycles at 95 ° C for 10 s, 60 ° C for 10 s and 72 ° C for 10 s. To compare mRNA level, relative quantification was performed as outlined in Pfaffl et al. Normalization was realized thanks to TBP and CyclophilinA housekeeping genes. Total RNA was extracted and analyzed by qRT-PCR for the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Stearoy-CoA desaturase (SCD1), Acyl-CoA oxidase 1 (ACOX1), Lipoprotein lipase (LPL), Carnitine palmitoyl transferase 1 (mCPT1), (Uncoupling protein 1) UCP1, Protein kinase AMP-activated (AMPK), , Fatty transporter CD36 FAT (CD36), Fatty Acid Synthase (FASn). Displayed values are means ± SEM. (n=5-6). *p<0,05 NaCl vs TG. Primers sequences are listed in a separate table in supplementary material (see Table S1).

Supplementary table. List of primers used

UCP1	**5'-**GGCCTCTACGACTCAGTCCA **-3'** **5'-**TAAGCCGGCTGAGATCTTGT **-3'**
mCPT1	**5'-** CAGCTGGCTGGTTGTTGTCA**-3'** **5'-** TTGTCGGAAGAAGAAAATGC**-3'**
Acox1	**5'-**GCCCAACTGTGACTTCCATC **-3'** **5'-**GCCAGGACTATCGCATGATT **-3'**
SCD1	**5'-**TTCCCTCCTGCAAGCTCTAC**-3'** **5'-**CAGAGCGCTGGTCATGTAGT **-3'**
GAPDH	5'- CCATGTTTGTGATGGGTGTGAA -3' 5'-GCCCTTCCACAATGCCAAA-3'
FASn	**5'-** GCTGCTGTTGGAAGTCAGC **-3'** **5'-** AGTGTTCGTTCCTCGGAGTG **-3'**
CD36	**5'-**TCTGACATTTGCAGGTCTATCT**-3'** **5'-**TGTCTGGATTCTGGAGGGGTGATGC **-3'**

AMPK	**5'-**CCTTCGGGAAAGTGAAGGT **-3'** **5'-**GAATCTTCTGCCGGTTGAGT **-3'**
LPL	**5'-**CACAGTGGCCGAGAGCGAGAA**-3'** **5'-**GCTGAGTCCTTTCCCTTCTGCAG **-3'**
Cyclophilin A	**5'-** ACGCCACTGTCGCTTTTC -**3'** **5'-** GCAAACAGCTCGAAGGAGAC **-3'**
TBP	**5'-** GGGGAGCTGTGATGTGGAAGT **-3'** **5'-** CCAGGAAATAATTCTGGCTCA**-3'**

SUPLEMENTARY REFERENCES

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