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ORIGINAL ARTICLE

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

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Circulating triglycerides (TGs) normally increase after a meal but are altered in pathophysiological conditions, such as obesity. Although TG metabolism in the brain remains poorly understood, several brain structures express enzymes that process TG-enriched particles, including mesolimbic structures. For this reason, and because consumption of high-fat diet alters dopamine signaling, we tested the hypothesis that TG might directly target mesolimbic reward circuits to control reward-seeking behaviors. We found that the delivery of small amounts of TG to the brain through the carotid artery rapidly reduced both spontaneous and amphetamine-induced locomotion, abolished preference for palatable food and reduced the motivation to engage in food-seeking behavior. Conversely, targeted disruption of the TG-hydrolyzing enzyme lipoprotein lipase specifically in the nucleus accumbens increased palatable food preference and food-seeking behavior. Finally, prolonged TG perfusion resulted in a return to normal palatable food preference despite continued locomotor suppression, suggesting that adaptive mechanisms occur. These findings reveal new mechanisms by which dietary fat may alter mesolimbic circuit function and reward seeking.

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INTRODUCTION

The regulation of energy balance depends on the ability of the brain to provide an adaptive response to changes in circulating factors of hunger and satiety.[1–3] The hypothalamus is regarded as a key integrative structure in the homeostatic control of energy balance, but feeding behavior is also modulated by sensory input (for example, tastes and odors) and emotional state.[4–8] Calorie-dense foods and other objects of desire (for example, sex, drugs) stimulate dopamine (DA) release, a key signal underlying their potent reinforcing properties.[9–12] In particular, the projection of DA neurons in the ventral tegmental area to the nucleus accumbens (NAc) is a crucial substrate upon which drugs of abuse exert their effect and thus this projection is often referred to as the brain's 'reward circuit'. As with drugs of abuse, this projection is a substrate upon which calorie-rich foods act to promote positive reinforcement, and adaptations in this circuit may underlie the downward spiral of uncontrolled craving, compulsive overconsumption and ultimately obesity.[7,12–15]

Indeed, multiple lines of evidence now suggest that obesity or chronic exposure to a high-fat high-sucrose (HFHS) diet is associated with deficits in the reward system.[11,14–19] For example, striatal levels of the dopamine D2 receptor (D2R) are inversely related to body weight (BW), and D2R knockdown rapidly accelerates the development of addiction-like reward deficits in rats with extended access to high-fat food.[14] In humans, obesity is associated not only with decreased striatal DA release in response to palatable food intake[16] but also with greater activation of the reward system during food reward anticipation.[11,15,16,19–21]

Furthermore, animals consuming high-fat diet, independent of obesity, exhibit decreased DA turnover in cortical and striatal structures, reduced preference for psychostimulants, attenuated operant responding for sucrose and cognitive deficits.[18] Moreover, obesity-associated cognitive impairment can be improved by selective lowering of circulating triglyceride (TG), while intracerebroventricular injection of TG impairs learning in normal mice.[22] Together, these observations raise the possibility that nutritional lipid, particularly TG, directly affect cognitive and reward processes.

Circulating lipids are found as non-esterified long-chain fatty acids (LCFAs) but also esterified and packaged into TG-rich particles such as chylomicrons or liver-born very low density lipoprotein. TG and LCFAs are both circulating lipid species, but their physiological entries into plasma differ drastically. LCFA typically increase in response to a fast due to adipose lipolysis——whereas TG-rich particles accumulate after a meal.[23] Although LCFA metabolism at the level of the hypothalamus regulates energy balance,[24–29] little is known about brain TG sensing.

Both mesolimbic and hypothalamic regions express enzymes involved in the transport, manipulation and metabolism of TG.[30–37] In particular, the hippocampus and the NAc express lipoprotein lipase (LpL), a key enzyme involved in TG hydrolysis.[38] Recent studies have highlighted the importance of brain TG hydrolysis in the regulation of energy balance,[39] and the variation in circulating TG after a high-fat meal was a strong predictor for hyperphagia and obesity,[40] but until now a physiological model

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that allows for the study of TG action on the brain has been missing.

Here we describe a novel physiological model in which freely moving mice receive small amounts of TG emulsion infused through the carotid artery in the direction of the brain. Without affecting circulating lipids or BW, brain TG delivery rapidly reduced spontaneous and amphetamine-induced locomotion. Brain TG delivery also decreased reward seeking, whereas NAc-selective knockdown of LpL increased reward seeking. Finally, brain TG delivery abolished preference for palatable food, whereas NAc-specific LpL knockdown increased palatable food intake. Based on these data, we conclude that LpL-mediated hydrolysis of nutritional TG within the mesolimbic system modulates reward impact.

MATERIALS AND METHODS

Animals and diets
All animal experiments were performed with approval of the Animal Care Committee of the University Paris Diderot-Paris 7. Ten-week-old male mice C57Bl/6 J (25–30 g, Janvier, Le Genest St Isle, France) were housed individually in stainless steel cages in a room maintained at $22 \pm 3\,°C$ with light from 0700–1900 hours. Eight to 10 week old animals C57Bl/6 J were subjected to a 6-month high-fat feeding to produce diet-induced obese (DIO) animals (see Supplementary Information).

Catheter-implantation procedures
Mice were anesthetized with isoflurane and received $10\,\mu g\,kg^{-1}$ intraperitoneal (i.p.) xylazine (Rompun) for the insertion of a catheter in the carotid artery towards the brain. Then the catheter was exteriorized at the vertex of the head.[41] Following surgery, mice received $10\,\mu g\,kg^{-1}$ i.p. ketoprofen (Ketofen). Catheters were kept patent through daily flushing with a solution of NaCL+ heparin ($20\,U\,ml^{-1}$). Infusions started after a recovery period of 7–10 days.

Infusion procedures
After a 10-day recovery period, catheters were connected to a swiveling infusion device, allowing the animal free movement and access to water and food. Mice were infused with saline solution (0.9% NaCl, $0.1–0.5\,\mu l\,min^{-1}$) for 3–4 days for habituation to the infusion device. Mice were then divided into two groups: one continuing to receive saline (NaCl mice) and the other receiving a TG emulsion (TG mice) (intralipid) at a rate of $0.1–0.3\,\mu l\,min^{-1}$ for the next 4 days or for 6h prior amphetamine test of progressive ratio (PR) test. Due to small variations in catheters, we varied the pump settings slightly across animals to obtain constant flow rates of $\sim 0.1\,\mu l\,min^{-1}$ at the exit of the tube.

Viral production
The plasmid CBA.nls myc Cre.eGFP expressing the myc-nls-Cre-GFP (green fluorescent protein) fusion protein[42] was kindly provided by Richard Palmiter (University of Washington, Seattle, WA, USA). Adeno-associated virus serotype 2/9 (AAV2/9) (6×10^{11} genomes ml^{-1} and 1.7×10^{8} infectious units μl^{-1}) were produced by the viral production facility of the UMR INSERM 1089 (Nantes, France).

Stereotaxic procedures
Mice were anesthetized with isoflurane and received $10\,\mu g\,kg^{-1}$ i.p. xylazine analgesic. They were then placed on a stereotaxic frame. AAV Cre-GFP virus were injected bilaterally into the NAc (stereotactic co-ordinates are relative to bregma: X:± 1 mm; Y:+1.5 mm; Z:-4.2 mm) at a rate of $0.20\,\mu l\,min^{-1}$ for 5 min per side. At the end of surgical procedures, mice received $10\,\mu g\,kg^{-1}$ i.p. ketoprofen.

Measurement of locomotor activity and food intake
Metabolic analyses were performed in an automated online measurement system using high sensitivity feeding and drinking sensors and an infrared beam-based activity monitoring system (Phenomaster, TSE Systems GmbH, Bad Homburg, Germany).

Amphetamine injection
After 6 h of TG infusion, mice were given i.p. injection of saline on the first 2 days, and $3\,mg\,kg^{-1}$ i.p. D-amphetamine (A5880, Sigma-Aldrich, L'Isle d'Abeau, France) on the subsequent 2 days. Locomotor activity for each animal was measured during 2 h after vehicle or amphetamine injection.

Operant conditioning system
Computer-controlled operant conditioning was conducted in 12 identical conditioning chambers equipped with a swiveling infusion device (Phenomaster, TSE Systems GmbH). Each chamber contains an operant wall with a food cup, two levers located 3 cm lateral to the food cup, with the left lever designated the active lever (for food pellet delivery). To ensure responding, animals were food restricted to 90% of initial BW. The reinforcer was a single 20-mg peanut butter-flavored sucrose tablet (TestDiet, Richmond, IN, USA).

LpL activity assay
Heparin-releasable LpL activity was assayed in microdissected brain regions using a kit (RB-LPL, Roar Biomedical, Inc., New York, NY, USA).

Statistical analysis
Displayed values are means $\pm$ s.e.m. Variance equality was analyzed by a F-test (Microsoft Excel, Issy-Les-Moulineaux, France). Comparisons between groups were carried out using a parametric Student's t test or by a non-parametric Mann–Whitney–Wilcoxon's test (Minitab, Paris, France). When appropriate, repeated measures analysis were performed by using General Linear Regression analyses followed by a Bonferroni's test with time (day or min) and groups and their interaction as factor (Minitab). A P-value of < 0.05 was considered statistically significant.

RESULTS

Central TG delivery decreases locomotor activity and abolishes preference for palatable food
To assess whether TG-rich particles act as brain signaling molecules, we established a model of brain-specific infusion of phospholipid TG emulsion (Intralipid 20%, $2\,kcal\,ml^{-1}$) through the carotid artery in the direction of the brain in freely moving animals (Supplementary Figures S1a and b). To test whether TG delivered via this route is metabolized in the brain, we infused radiolabelled TG ([9,10-^{3}H(N)]-triolein) through the carotid artery. Detectable quantities of radiolabelled LCFA were present in brain structures known to express TG-processing enzymes indicative of local TG hydrolysis (Supplementary Figure S2). Intralipid was infused at a rate of $0.1–0.3\,\mu l\,min^{-1}$ to approximate the physiological amount of TG entering the brain (see Materials and methods). Importantly, at this rate of infusion 24-h intra-carotid TG infusion does not affect circulating levels of free fatty acid, TG or cholesterol in the periphery (Supplementary Figure S1c).

Next we assessed the behavioral and metabolic consequences of brain TG delivery using modified home cages allowing for chronic infusion while measuring food consumption, water intake and locomotor activity. After catheter implantation, mice were allowed to recover for 10 days followed by 4 days of saline infusion; during this time, baseline parameters were acquired, mice were split into saline- and TG-infused groups and presented with choice of normal chow and HFHS diet (Figure 1a). Although both groups were indistinguishable during basal saline/chow condition (not shown), TG perfusion selectively decreased nocturnal, but not diurnal, locomotor activity (Figures 1b and c). Importantly, this action of TG on locomotion rapidly diminished when TG infusion was discontinued, and animals were returned to saline infusion (Supplementary Figure S3). Consistent with the reduction in activity, we found a decrease in energy expenditure at several time points of the dark period during TG infusion, as well a decrease in nocturnal respiratory quotient (Supplementary Figure S4).

On food choice, saline-infused animals clearly showed a strong preference for the palatable diet over chow; however, mice
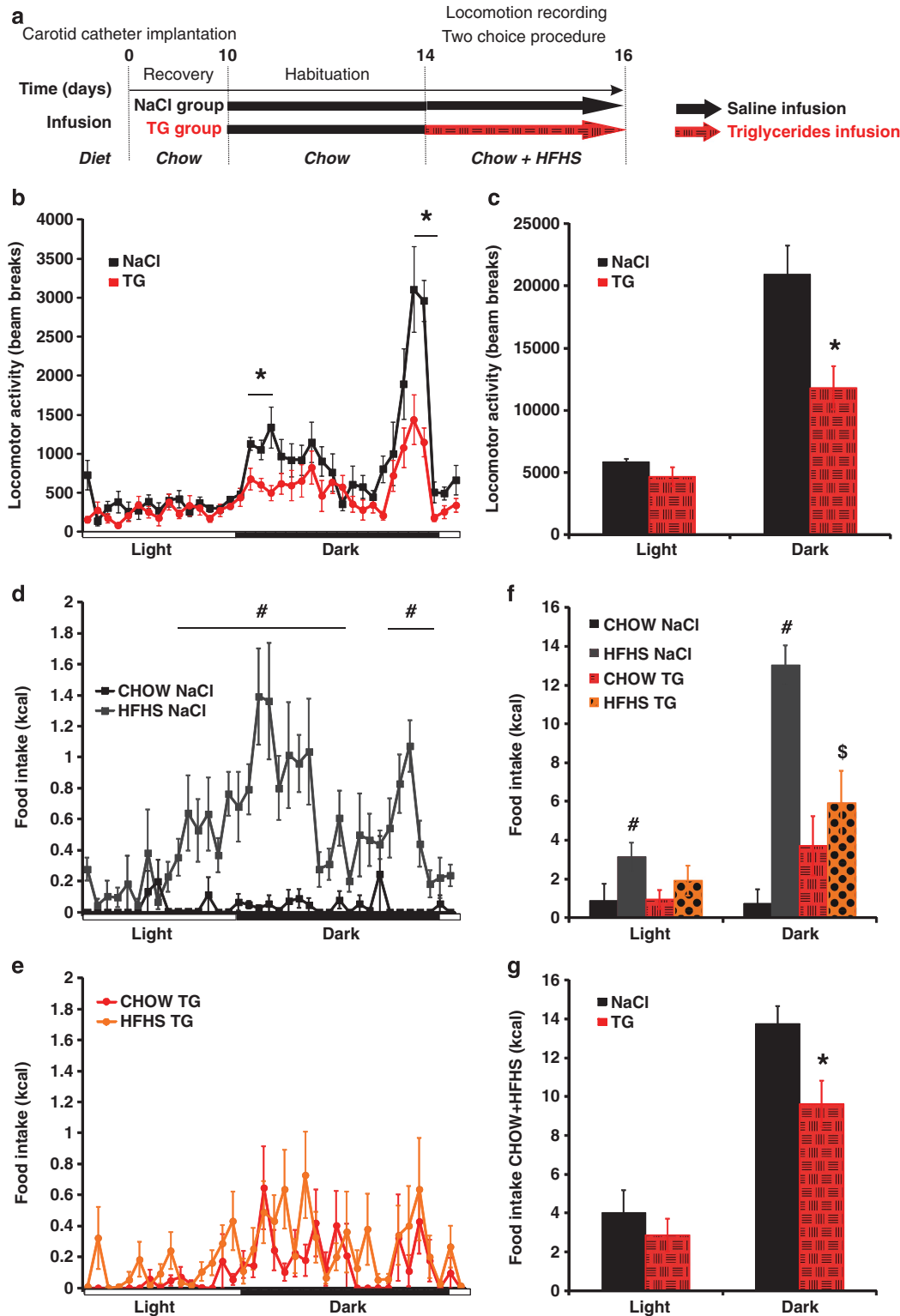
Figure 1. Central triglyceride (TG) delivery specifically decreases nocturnal locomotor activity and abolishes feeding preference for palatable food. Experimental procedure (**a**). Daily variation of locomotor activity (**b**) and cumulative locomotor activity during light and dark periods (**c**) in control mice (black squares and bars) and TG-infused mice (red circles and hatched red bars). Daily variation in chow (black squares) and high-fat high-sucrose (HFHS; gray squares) diet intake in control mice (**d**). Daily variation in chow (red circles) and HFHS (orange 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 red bars) and HFHS diet (control mice: grey bars; TG-infused mice: dotted orange bars) (**f**). Cumulative total food intake (chow+HFHS diet) during light and dark periods in control mice (black bars) and TG-infused mice (hatched red bars) (**g**). Locomotor and feeding recording period are indicated by an hatched arrow. Data present the mean of days 14+15. Displayed values are means ± s.e.m. (*n* = 5-6). *$P < 0.05$ NaCl vs TG; #$P < 0.05$ CHOW NaCl vs HFHS NaCl; $$P < 0.05$ HFHS NaCl vs HFHS TG.
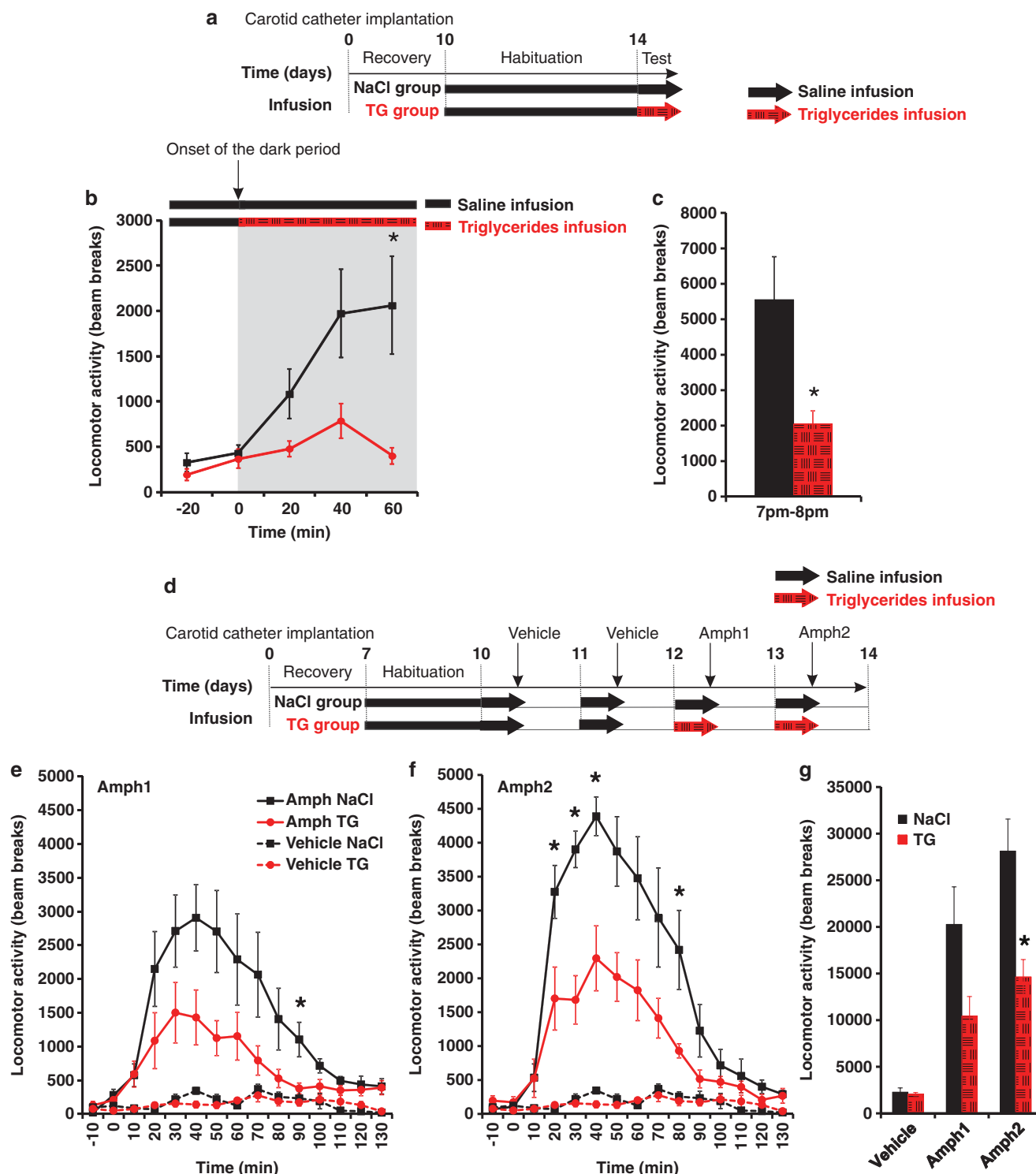
Figure 2. Central triglyceride (TG) delivery rapidly alters spontaneous locomotor activity and decreases amphetamine-induced locomotion. Experimental procedure (**a**, **d**). Locomotor activity evolution (**b**) and cumulative activity (**c**) was monitored at the very beginning of the infusion at the onset of dark period (1900–2000 hours) in control mice (black squares and bars) and TG-infused mice (red circles and hatched red bars). Locomotor activity was recorded during 2 h after an acute intraperitoneal injection of 3 mg kg^{-1} D-amphetamine or vehicle (saline solution) in control mice (black square) and TG-infused mice (red circles) on day 12 (**e**) and day 13 (**f**). Cumulative locomotor activity after vehicle or amphetamine injection in control mice (black bars) and TG-infused mice (hatched red bars) (**g**). Mice were infused with NaCl or TG solution during 6 h just before vehicle or D-amphetamine injection. Displayed values are means ± s.e.m. ($n = 5$). *$P < 0.05$ NaCl vs TG.

receiving TG consumed a similar amount of calories from both diets (Figures 1d–f), indicating that brain TG delivery abolished preference for palatable food with little effect on total caloric intake (chow+HFHS) (Figure 1g). Importantly, when animals were

not given the choice between diet, central TG still reduced locomotor activity but did not alter normal chow intake, ruling out potential adverse responses to centrally delivered TG (Supplementary Figure S5).
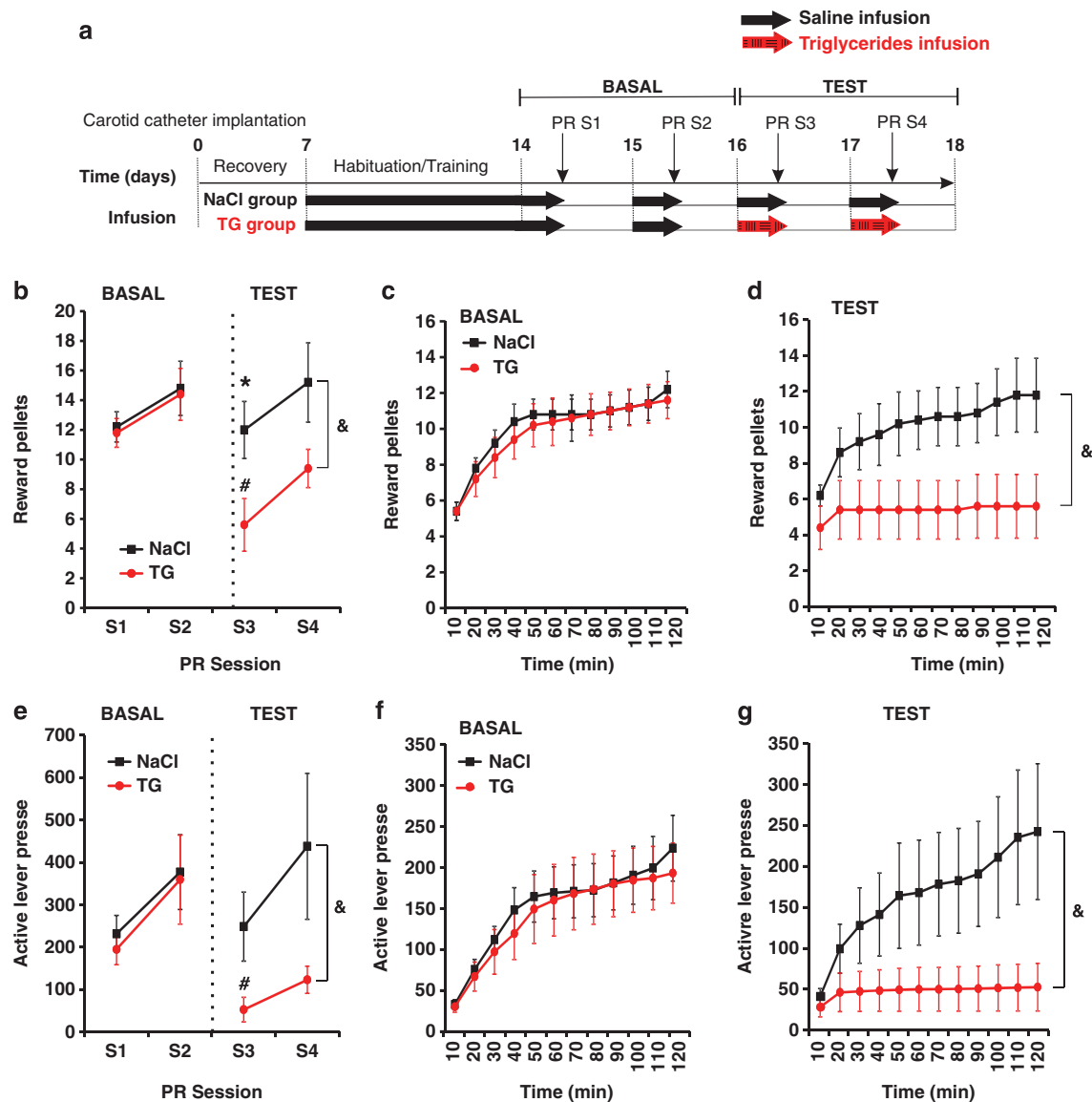
Figure 3. Central triglyceride (TG) delivery specifically decreases motivational aspect of reward seeking. Experimental procedure (a). Progressive ratio (PR) responding for sucrose pellet in control mice (black squares) and TG-infused mice (red circle). During basal conditions, both group were infused with saline solution. Numbers of rewards achieved (b) and active lever presses achieved (e) were recorded during four sessions of PR. Time course of number of rewards and active lever presses were recorded during the first (c, f) and third PR session (d, g). Mice were infused with NaCl or TG solution during 6 h just before the session of PR. Displayed values are means $\pm$ s.e.m. ($n = 5$). *$P < 0.05$ NaCl vs TG; #$P < 0.05$ TG S1 and S2 vs TG S3. Using repeated measure analysis, we show an effect of group (NaCl vs TG) during TEST, &$P < 0.001$.

Central TG delivery decreases spontaneous and amphetamine-induced locomotion

The sharp reduction in nocturnal locomotor activity prompted us to explore the effects of TG perfusion on the increase in spontaneous activity that normally occurs at the onset of the dark period. Animals received saline perfusion up until 20 min before the initiation of the dark cycle at which point one group was shifted to TG perfusion (Figure 2a). Although lights off triggered a 400% increase in locomotion in the saline-infused control group, the group receiving TG remained at a level only slightly above diurnal level (Figures 2b and c). This result indicates that brain-specific TG delivery can act rapidly to alter behavior. Both spontaneous activity and preference for palatable food are regulated by the release of DA in striatal and limbic brain regions.[17,18,43–45] We therefore reasoned that TG entering the brain may influence DA signaling and tested the ability of

amphetamine to induce locomotion in animals receiving a short 6-h intra-carotid perfusion of TG (Figure 2d). Central TG delivery reduces amphetamine-induced locomotion by ~50% (Figures 2e and f), suggesting a potential role for nutritional TG in the regulation of DA signaling. Again, upon cessation of TG infusion, animals regained sensitivity to amphetamine (Supplementary Figure S6). In addition, we recapitulated these results using the selective D2R agonist quinpirole. At a dose (0.1 mg kg^{-1}) shown to acutely increase locomotion,[46] central TG delivery decreased quinpirole-induced locomotion (Supplementary Figure S7).

Evaluation of DA and DA metabolites 2-h after amphetamine injection revealed that central TG delivery was associated with a modest increase in striatal DA turnover (Supplementary Figures S8a–e). However, in the absence of acute amphetamine, 24-h TG perfusion was insufficient to alter DA turnover (Supplementary Figures S8f and i). Similarly, we found no significant changes in the levels of striatal D2R protein
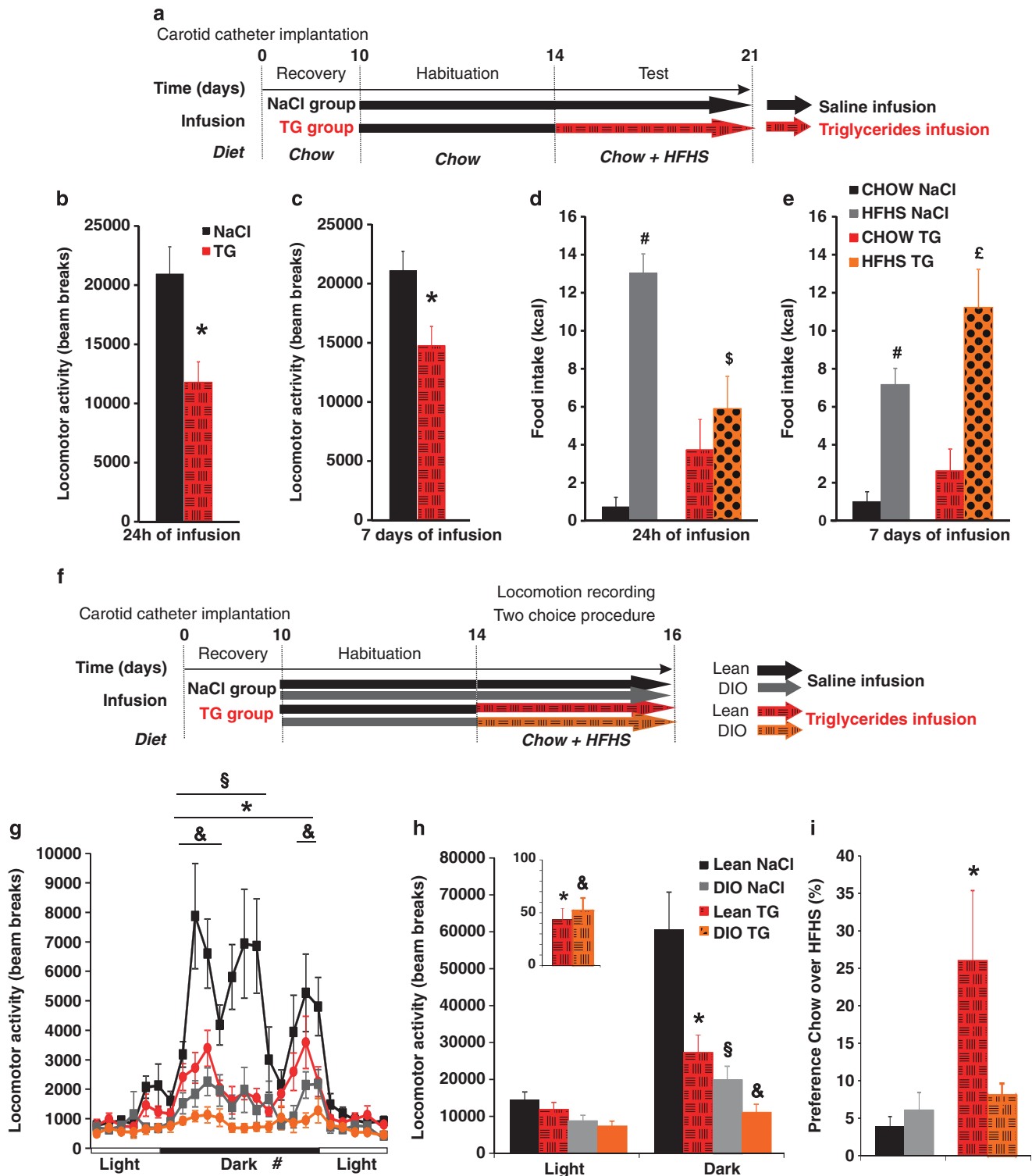
Figure 4. Prolonged central triglyceride (TG) delivery results in desensitization of feeding but not locomotor activity. Experimental procedure (**a**, **f**). Cumulative locomotor activity during dark period in control mice (black bars) and TG-infused mice (hatched red bars) was recorded after 24 h (**b**) or 7 days (**c**) infusion. Cumulative food intake during dark period of chow (control mice: black bars; TG-infused mice: hatched red bars) and high-fat high-sucrose (HFHS) diet (control mice: grey bars; TG-infused mice: dotted orange bars) was recorded in a food choice procedure after 24 h (**d**) or 7 days (**e**) of infusion. Daily variation of locomotor activity (**g**), cumulative locomotor activity (**h**) (insert represents the reduction of locomotor activity expressed in percentage of total activity during saline perfusion) and ratio between chow/HFHS kcal intake during a two-choice procedure (**i**) in lean mice infused with NaCl (black squares and bars), in lean mice infused with TG (red circles and hatched red bars), in diet-induced obese (DIO) mice infused with NaCl (grey square and bars) and in DIO mice infused with TG (orange circles and hatched orange bars). Data present the mean of days 14+15 (**b**, **d**, **g**, **h**) and days 19+20 (**c**, **e**). Displayed values are means ± s.e.m. ($n = 5$–7). *$P < 0.05$ NaCl vs TG; #$P < 0.05$ CHOW NaCl vs HFHS NaCl; $^\$P < 0.05$ HFHS NaCl vs HFHS TG; $^£P < 0.05$ CHOW TG vs HFHS TG; $^\&P < 0.05$ NaCl-DIO vs TG-DIO; $^\S P < 0.05$ DIO vs Lean.

(Supplementary Figure S9) or enkephalin (a peptide enriched in D2R-expressing striatal neurons and involved in food reward[5,47]) (Supplementary Figure S10). Taken together, these data suggest that TG may act within the striatum, downstream of DA receptor binding, to alter basal ganglia output.

Central TG delivery decreases food reward seeking

Palatability and hedonic impact are powerful determinants of food intake. Ingestion of food, particularly foods rich in sugar or fat, provides subjective pleasure and can be a source of comfort in response to depression or stress.[4,9,11,48,49] The action of central TG delivery on food preference, nocturnal activity and psycho-stimulant-induced locomotion suggested that brain lipid signaling may directly influence motivated behavior. We tested this hypothesis by first pretraining mice to lever press for high-sucrose food reward pellets, and then following intra-carotid saline or TG infusion, assessing their performance on a PR task that measures the amount of effort an animal is willing to exert to obtain food rewards (Figure 3a).

Mice received saline for the first two consecutive PR sessions (S1 and S2) and were then divided into two groups receiving either saline or TG for an additional two consecutive sessions (S3 and S4). Although both groups demonstrated similar performance during S1 and S2 (basal saline infusion), 6-h TG delivery was sufficient to induce a significant decrease in lever presses and rewards received during S3 and S4 (Figures 3b–g). Importantly, there were no changes in the ratio of active vs inactive lever presses, suggesting that TG infusion does not impair discrimination learning or memory retrieval in this task (Supplementary Figure S11). These results indicate that changes in circulating levels of nutritional TG are sensed within the brain and are sufficient to suppress reward-seeking behavior in the absence of any changes in metabolic demand.

In conditions of chronic hypertriglyceridemia, central TG delivery no longer affects feeding preference but still reduces locomotor activity

Our results suggest that an immediate surge in plasma TG after a meal would act in the brain to inhibit preference and desire for high-fat or high-sucrose foods and thereby homeostatically regulate feeding behavior. However, chronic hypertriglyceridemia and altered feeding behavior, particularly intense craving for highly palatable food, are symptoms commonly associated with obesity.[16,23] In order to reconcile these observations, we compared the behavioral consequences of 1 vs 7 days of brain TG delivery. Mice received either saline or TG infusion in the carotid artery, and locomotor activity, food intake and food choice were continuously monitored for the first 24 h and again on the seventh day of infusion (Figure 4a). Importantly, even if animals had a slight increase in BW due to HFHS diet exposure, they were not obese. Results show that brain-specific TG infusion was equally effective in reducing locomotor activity on day 1 or day 7 (Figures 4b and c). However, while TG delivery suppressed preference for palatable food on day 1, by the seventh day of TG infusion mice displayed a normal preference, suggesting that adaptive mechanisms had occurred (Figures 4d and e). Importantly, over the course of infusion BW remained unchanged (Supplementary Figure S12), arguing against non-specific effects of these procedures on animal health. Because obesity leads to chronically elevated TG, the action of centrally delivered TG was also evaluated in DIO mice (36.5 g ± 0.99 vs lean 26 g ± 0.26 g of BW, $P < 0.05$) using a protocol similar to that in Figure 1 (Figure 4f). In agreement with previous observations,[50] DIO mice exhibited reduced locomotor activity compared with lean counterparts under baseline conditions. Consistent with our hypothesis, central TG delivery in lean mice induces a decrease in activity to the level of saline-perfused DIO (Figures 4g and h) while TG perfusion in both lean and DIO mice

similarly induced a ∼ 50% decrease in locomotor activity (Figures 4g and h and insert). Central TG delivery also reduced amphetamine-induced locomotion by ∼ 50% in both lean and DIO mice (Supplementary Figure S13). Finally, using the food choice procedure, central TG delivery increased the consumption of chow over HFHS food in lean mice as we had previously observed but did not affect tropism for palatable food in DIO mice (Figure 4i).

NAc-specific Lpl knockdown increases reward seeking and palatable food consumption

Our data using brain TG infusion suggest that TG-sensing neural circuits detect changes in circulating TG to alter behavior. The behavioral consequences suggest a role for mesolimbic circuits, but the molecular mechanisms of TG sensing remain obscure. In the hypothalamus, LpL-mediated TG hydrolysis has an important role in TG sensing, and suggestively, this enzyme is also expressed at high levels in striatum.[30,34,37,39] We therefore hypothesized that LpL-mediated TG hydrolysis locally in the NAc acts as molecular relay for TG effects on reward seeking. Consistent with these findings, in situ hybridization confirmed the expression of Lpl mRNA in NAc neurons, (Figures 5a and b) with significantly lower expression levels across cortical regions (Figure 5c), highlighting the specificity of Lpl expression and supporting a functional role in NAc. LpL activity is also present in several brain structures, including the hypothalamus, the hippocampus, the striatum and the cortex (Supplementary Figure S14a); and consistent with mRNA expression levels, LpL activity was higher in NAc than in cortex. We next engaged in the selective knock down of LpL in the NAc by stereotactic injection of AAV expressing a chimera of Cre recombinase fused to GFP[42] (AAV-Cre:GFP) into the NAc of mice in which the first exon of Lpl is flanked by LoxP sites (Lpl^{lox/lox}), allowing for Cre-mediated Lpl gene deletion[39,51] (see Figure 5d and Supplementary Figures S14b and d). Following AAV-Cre:GFP injection into the NAc, LpL activity was decreased by ∼ 30% in the NAc of Lpl^{lox/lox} compared with control Lpl^{+/+} mice (referred as to NAc-Lpl^{ΔΔ} and NAc-Lpl^{++}) (Figure 5e).

NAc-Lpl^{ΔΔ} and NAc-Lpl^{+/+} mice were subjected to the operant task described above. NAc-specific knock down of LpL activity did not impact performance on a low fixed ratio task during pretraining (not shown). However on a PR task, NAc-Lpl^{ΔΔ} displayed an increase in both rewards received and lever presses compared with NAc-Lpl^{+/+} (see Figures 5f–h), with no effect on the ratio of active/inactive lever presses (Figure 5i). Thus while brain TG delivery decreased reward-seeking behavior, NAc-specific knock down of the TG processing enzyme LpL had the opposite effect, increasing the drive to work for rewards (Figure 5). These data are consistent with a role for LpL-mediated TG signaling in the NAc in the regulation of food-seeking behavior.

Because intra-carotid delivery of TG abolished preference for HFHS diet (Figure 1), we predicted that NAc-specific reduction of LpL activity might produce the opposite outcome. Cumulative food intake was monitored in NAc-Lpl^{+/+} and NAc-Lpl^{ΔΔ} mice during ad libitum feeding with regular chow and over a 24-h period during which regular chow and HFHS diet were available. NAc-specific inactivation of LpL had no impact on regular chow consumption (Figure 5j). However, when given the choice, NAc-Lpl^{ΔΔ} were hyperphagic and consumed ∼ 30% more calorie-dense HFHS food compared with NAc-Lpl^{+/+} mice (Figure 5k). This result indicated that impairing the ability to hydrolyze TG specifically in the NAc resulted in increased incentive to consume palatable food. Taken together, these results indicate that nutritional TG, detected directly at the level of the NAc, modulate the reinforcing properties of palatable food.

DISCUSSION

The past several decades have witnessed a pandemic expansion of pathologies related to high-fat and high-carbohydrate diets,
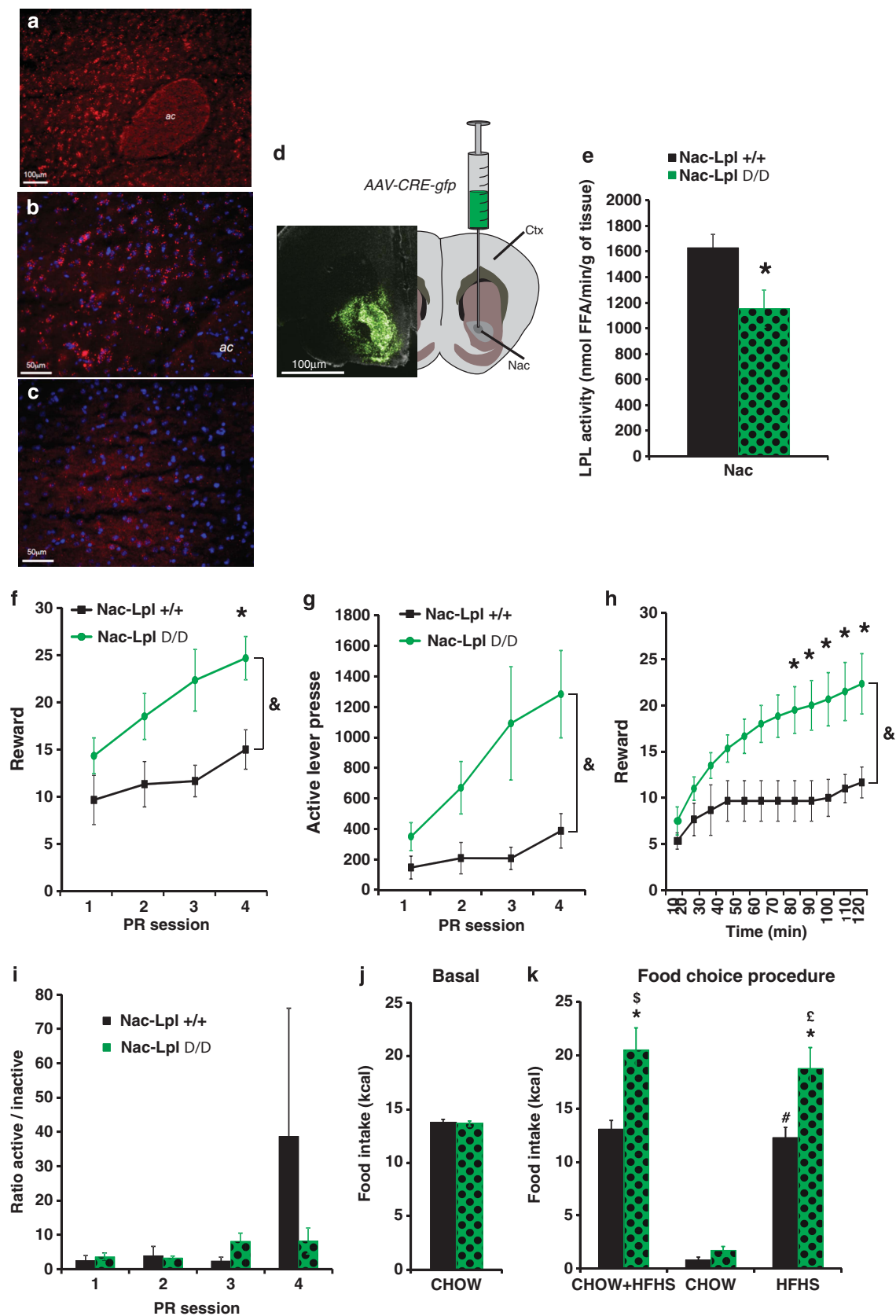
including obesity, diabetes, dyslipidemia and cardiovascular diseases—collectively referred to as metabolic syndrome.[52,53] Globally, there has been both an increased intake of energy-dense foods, which are high in fat, sugar and salt, and a concomitant decrease in physical activity.[52,53] Despite the increasing prevalence and burden of the metabolic syndrome, effective and safe treatments to reverse the epidemic remain unavailable.

Although the presence of high-fat nutrient-rich food is essentially ubiquitous in developed countries, not all individuals with access to these foods overconsume them or become obese. It is thus likely that certain individuals become more vulnerable to the hedonic or reinforcing effects of calorie-rich food and ultimately form eating habits that become disassociated from the homeostatic mechanisms that normally maintain energy balance.[3] Understanding the early mechanisms underpinning the progressive loss in homeostatic control of energy balance is a key step toward a clinical breakthrough in the treatment of hyperphagia and obesity.

Hypothalamic and extra hypothalamic lipid sensing: different lipid species for a different purpose?

LCFA metabolism at the level of the hypothalamus has been shown to regulate neuronal activity, food intake, autonomic control of insulin release and liver insulin-sensitivity.[24–27,54] But although TG and LCFAs are both circulating lipid species, the physiological entry into plasma differ drastically. Indeed, whereas TG-rich particles accumulate after a meal; LCFA are released by fasting-induced adipose lipolysis and are thus elevated during periods of food abstinence.[23] In addition, while fatty-acid transporters promote the uptake of LCFA into various brain structures, TG must first be hydrolyzed by specific TG-hydrolyzing enzymes, the distribution of which will severely limit central availability of TG-derived signals. Hence, the central structures involved in the detection of peripheral lipid fluctuations as well as the nature of the adaptive behavioral and metabolic responses might radically differ depending on the lipid species and the physiological status (for example, fasting, postprandial or obesity-associated hypertriglyceridemia).

Previous work on how circulating lipids affect the brain has focused chiefly on the effects of non-esterified fatty acids on brain metabolism and behavior. For instance, intracerebroventricular injection of oleic acid was shown to alter hypothalamic lipid metabolism and result in decreased food intake.[24] Furthermore, lipid metabolism in the hypothalamus has been repeatedly shown to be critical in mediating the central effects of LCFAs.[24–27,54,55] However, 48-h intra-carotid TG delivery did not alter whole brain expression levels of key lipid-modifying or lipid-sensitive enzymes (Supplementary Figure S15). Omega-3 polyunsaturated fatty acids have anti-inflammatory properties, which could contribute to the central effects of TG.[56] However, intra-carotid infusion of a TG emulsion enriched in the omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid recapitulated the behavioral effects on food choice and locomotor activity, suggesting that the pro- or anti-inflammatory nature of omega-3 vs omega-6 lipids were not fundamentally involved in the behavioral phenomena we observed (Supplementary Figure S16).

We therefore propose that the central availability of TG and LCFA differ, that these lipid species influence neural circuit function through separate mechanisms, and that they have opposing effects on food-seeking behavior. Although LCFAs may principally act in the hypothalamus and function to increase food intake in response to a fast, TG sensing may depend on local hydrolysis by LpL in limbic structures where they decrease the incentive or motivational properties of food. Potential mechanisms could involve lipid-mediated activation of membrane receptors,[57] cellular energy-related pathways,[27,54] endoplasmic reticulum stress,[58] eicosanoids-dependent inflammatory processes,[59] endocannabinoid signaling pathways[60,61] and lipid-activated transcriptional adaptations.[62]

Central detection of TG and the modulation of reward

Our results provide the first experimental evidence that TG can directly target mesolimbic structures to modulate reward seeking. We have established a method to evaluate the behavioral and metabolic consequences of brain lipid sensing by infusing TG via the natural route of access to the brain through the carotid artery (1) in the direction of the brain, (2) at a rate and concentration that closely recapitulate the postprandial increase in TG but (3) without affecting systemic LCFA or TG concentrations. Such TG infusion leads to a decrease in nocturnal and amphetamine-induced locomotor activity in both normal (lean) and DIO mice but only abolishes preference for palatable HFHS food only in lean mice. In addition, brain TG delivery decreases the overall motivation to work for reward as assessed by a PR task. We have also begun to define the neural circuitry and molecular mechanisms by which TG influence neural activity and behavior. Indeed, in contrast to the effects of TG infusion, deletion of the gene encoding the TG-hydrolyzing enzyme LpL specifically in the NAc leads to sensitization to the reinforcing properties of palatable food as revealed by the PR operant task and hyperphagia during a HFHS food preference task.

Plasma TG accumulate after a meal and gradually return to basal levels due to the contribution of both insulin-mediated TG storage and lipid oxidation in tissue.[23] However, plasma TG is chronically elevated in obesity. During prolonged TG perfusion, designed to mimic chronic hypertriglyceridemia, we found that adaptive desensitization processes occur such that TG infusion no longer abolishes HFHS preference but continues to suppress locomotor activity (Figure 4). Consistent with this, using DIO mice to physiologically model chronic hypertriglyceridemia, central TG delivery still reduced locomotor activity but no longer was effective in modulating food tropism (Figures 4f–i).

Figure 5. Nucleus accumbens (NAc)-specific lipoprotein lipase (LpL) knock down increases motivational aspect of reward seeking. Fluorescent *in situ* hybridization was performed for LpL mRNA (red) within NAc (**a**) revealing that the gene is expressed across multiple NAc neurons labeled with DAPI (blue, **b**). No expression of LpL mRNA was observed in white matter tracks (*anterior commissure, ac*) or other brain regions, as reflected in the absence of LpL mRNA grains within the somatosensory cortex (**c**). Schematic and representative photo micrograph showing green fluorescent protein (GFP) expression after AAV-CRE-GFP virus injection in the NAc of *Lpl^lox/lox* mice (**d**). LpL activity in NAc extract of *Lpl^+/+* (NAc-*Lpl^++* black bars) and *Lpl^lox/lox* mice (NAc-*Lpl^ΔΔ* green dotted bars) after viral injection (**e**). Progressive ratio (PR) responding for sucrose pellets in control mice (black squares and bars) and in mice with a NAc-specific LpL knockdown (green circles and green dotted bars). Numbers of rewards (**f**), active lever presses (**g**) and ratio of active/inactive lever presses achieved (**i**) were recorded during four sessions of PR. Time course of rewards acquisition in within the third PR session (**h**). Average chow intake was recorded at several time points during 2 months after NAc-specific AAV-Cre-GFP virus injection (**j**). Total cumulative food intake (chow+high-fat high-sucrose (HFHS)) and specific cumulative food intake of chow and HFHS diet was recorded in a 24-h food choice procedure (**k**) in control mice (black bars) and mice with a NAc-specific LpL knockdown (green dotted bars). Displayed values are means ± s.e.m. ($n = 5$–6 in each group). *$P < 0.05$ NAc-*Lpl^++* vs NAc-*Lpl^ΔΔ*; $^{$}P < 0.05$ CHOW NAc-*Lpl^++* vs CHOW+HFHS NAc-*Lpl^++*; $^{#}P < 0.05$ CHOW NAc-*Lpl^++* vs HFHS NAc-*Lpl^++*; $^{‡}P < 0.05$ CHOW NAc-*Lpl^ΔΔ* vs HFHS NAc-*Lpl^ΔΔ*. Using repeated measure analysis, we show an effect of group (NAc-*Lpl^++* vs NAc-*Lpl^ΔΔ*), $^{&}P < 0.001$.

Together, these data suggest a model whereby postprandial increases in plasma TG are hydrolyzed locally in the striatum where they alter striatolimbic circuitry to effect a reduction in locomotor activity and homeostatically reduce the incentive properties of calorie-rich HFHS foods. However, in the face of sustained elevations in plasma TG, achieved either by intra-carotid perfusion or DIO, the homeostatic mechanisms that normally serve to reduce the hedonic impact of HFHS foods break down. This model predicts a positive feedback loop whereby chronically high plasma TG, such as occurs in obesity, cripple the homeostatic mechanisms that curb food intake resulting in uncontrolled caloric consumption and reduced physical activity. Such a mechanism would serve to drive BW gain, whereas making weight loss more difficult.

Further studies will be required to uncover the cellular basis of LpL-mediated mesolimbic TG sensing, the downstream molecular events that occur following TG hydrolysis, and how these events alter the activity of striatolimbic neural circuits. Finally, our findings suggest new avenues to therapeutic intervention in obesity, obesity-associated pathologies and perhaps other disorders of compulsive reward seeking, such as drug addiction, which will require testing in disease models.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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