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Short-term high-fat feeding induces a reversible net decrease in synaptic AMPA receptors in the hypothalamus

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Abstract

Dietary obesity compromises brain function, but the effects of high-fat food on synaptic transmission in hypothalamic networks, as well as their potential reversibility, are yet to be fully characterized. We investigated the impact of high-fat feeding on a hallmark of synaptic plasticity, *i.e.*, the expression of glutamatergic α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) that contain the subunits GluA1 and GluA2, in hypothalamic and cortical synaptoneurosomes of male rats. In the main experiment (experiment 1), three days, but not one day of high-fat diet (HFD) decreased the levels of AMPAR GluA1 and GluA2 subunits, as well as GluA1 phosphorylation at Ser845, in hypothalamus but not cortex. In experiment 2, we compared the effects of the three-day HFD with those a three-day HFD followed by four recovery days of normal chow. This experiment corroborated the suppressive effect of high-fat feeding on hypothalamic but not cortical AMPAR GluA1, GluA2, and GluA1 phosphorylation at Ser845, and indicated that the effects are reversed by normal-chow feeding. High-fat feeding generally increased energy intake, body weight, and serum concentrations of insulin, leptin, free fatty acids, and corticosterone; only the three-day HFD increased wakefulness assessed via video analysis. Results indicate a reversible down-regulation of hypothalamic glutamatergic synaptic strength in response to short-term high-fat feeding. Preceding the manifestation of obesity, this rapid change in glutamatergic neurotransmission may underlie counter-regulatory efforts to prevent excess body weight gain, and therefore, represent a new target of interventions to improve metabolic control.

Keywords: High-fat diet; AMPA receptor signaling; Hypothalamus; Cortex; Synaptic plasticity; Sleep/wakefulness.

1. Introduction

Food rich in saturated fat impairs cognitive performance and compromises synaptic plasticity [1,2], *i.e.*, the activity-dependent modification of the strength or efficacy of transmission at preexisting synapses [3]. Considering that excess intake of high-calorie, sweet and fatty "hyperpalatable" food [4] is the most prominent driver of obesity [5,6], changes in neuronal function induced by fluctuations in the quantity and quality of nutrient supply are of high clinical relevance [7]. Notably, being obese is associated with an increased risk of cognitive decline and dementia [8,9].

Synaptic strength is homeostatically regulated via synaptic scaling in response to global activity changes in individual cells or across larger neuronal ensembles [4,10]. Synaptic scaling is brought about by processes that include morphological and functional changes at the synapse and are thought to counter potentially maladaptive effects of long-term synaptic plasticity [11,12]. A major mechanism underlying synaptic scaling and, hence, plasticity is the trafficking of postsynaptic glutamatergic α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) [13]. AMPARs are the main ionotropic glutamate receptors mediating fast excitatory synaptic transmission in the mammalian brain, and their regulated trafficking underlies activity-triggered changes in transmission [14]. The predominantly expressed AMPAR subunits in the forebrain, including hypothalamus and neocortex, are GluA1 and GluA2, while GluA3 and GluA4 are less abundant [15,16]. The number of AMPARs is proportional to the area of the postsynaptic density [17] and GluA1 phosphorylation at distinct sites is known to regulate function, stabilization, and activity-dependent plasticity of synaptic AMPARs at synapses [10,18]. Synaptic strength increases with the delivery of AMPARs to excitatory synapses, while synaptic depression is associated with their removal [19].

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Glutamatergic signaling is the major mode of neurotransmission in the hypothalamus [20], the main brain center integrating nutritional and hormonal signals [21,22]. Neurons in the hypothalamus, in particular in the ventromedial and arcuate nuclei, are sensitive to nutrients and gut-derived signals [23], indicating that dietary manipulations could affect glutamatergic signaling and plasticity in the hypothalamus [24]. Indeed, three days of high-fat diet (HFD) are sufficient to increase the excitatory drive to anorexigenic arcuate proopiomelanocortin (POMC) neurons, possibly to counteract HFD-induced increases in energy intake [25]. Also, fasting and feeding differentially regulate AMPAR trafficking in POMC, but not neuropeptide Y (NPY)/Agouti-related peptide (AgRP) neurons, with GluA2-containing, calcium-impermeable receptors dominating in the fasting state and an increase in the excitatory drive via GluA2-lacking, calcium-permeable AMPARs after 10 days of HFD [26].

We set out to assess global synaptic changes in the hypothalamus in response to a short-term increase in calorie intake – *i.e.*, before obesity develops. To this end, we determined the acute effect of high-fat in comparison to regular feeding on GluA1 and GluA2 subunits of AMPARs and the phosphorylation state of GluA1 in synaptoneurosomes, *i.e.*, preparations that enrich for synaptic proteins. We also tested the specificity to synaptoneurosomes by measuring GluA1 and GluA2 content along with GluA1 phosphorylation in the supernatant fraction. We hypothesized that high-fat feeding increases synaptic strength in the hypothalamus, but not in cortical brain regions, and, further, that these changes are reversible by returning animals to regular diet. Since wakefulness is associated with synaptic upscaling and sleep with downscaling of cortical synaptic strength [27,28], and considering links between sleep loss and metabolic impairments [29], we also took into account sleep/wake behavior.

2. Materials and methods

2.1. Animals

Experiments were conducted in male Wistar rats (RjHan:WI) aged 10–11 weeks and weighing 300–400 g at the start of experiments (Janvier, Le Genest-Saint-Isle, France). Animals were housed, and experiments were performed at controlled temperature (20 ± 2°C) and humidity (55 ± 10%), and a controlled 12 h/12 h light/dark cycle with light onset at 6 AM. Water and food were available *ad libitum*. Animals were routinely checked by laboratory staff. Failures to groom and/or loss of more than 20% body weight were set as criteria of potential sickness and lead to euthanasia. All procedures were approved by the Regierungspräsidium Tübingen, Baden-Württemberg (permit number MPV 1/17) and performed in accordance with the European Directive 2010/63/EU for animal experiments.

2.2. Experimental design and procedure

After their arrival at the central animal facility, all animals were fed 9% fat standard chow (Ssniff, V1534, Soest, Germany) and handled for seven days. Subsequently, food intake was assessed daily by weighing initial food supply and respective remains to calculate daily energy intake. Animals were weighed once a day at 18.00 h.

In *experiment 1*, 18 rats were separated into three groups of six rats/group. The rats of the control group were fed normal chow for five days. Animals of the HFD groups received normal chow for five days and were then fed an HFD containing 45% fat (D12451, Ssniff, Soest, Germany) for one day (HF1D) or three days (HF3D; Fig. 1A). In *experiment 2*, twelve rats were divided into two groups of six rats/group. Animals of the first group were fed in accordance with the HF3D group of experiment 1. Animals of the second group (recovery control group) after three days of HFD were returned to normal chow for four additional days (Fig. 1D). In both experiments, animals were videotaped for offline wakefulness/sleep analysis in the six hours before sacrifice (18.00–0.00 h, Fig. 1G). Wakefulness and sleep were assessed using standard visual examination [30]. Sleep was scored whenever the rat showed a typical sleep posture and stayed immobile for at least 10 s. If brief movements <5 s interrupted sleep epochs, continuous sleep was scored. This visual scoring approach has been shown in previous rodent studies to consistently match conventional EEG/EMG-based scoring by more than 92% [31–34].

At the end of both experiments, animals were deeply anesthetized with isoflurane (within 1 min) and sacrificed by cervical dislocation. The head was cooled in liquid nitrogen and the whole brain was removed. The left cortical hemisphere and the hypothalamus were dissected, and samples were immediately frozen in liquid nitrogen and stored at −80°C until later use for synaptoneurosome preparation. In experiment 1, blood samples were collected via cardiac puncture when the animal was sacrificed, and centrifuged at 3,000 × *g* for 10 min to obtain serum, followed by storage at −80°C until further analysis.

2.3. Biochemical analyses

2.3.1. Synaptoneurosome preparation

Cortical and hypothalamic tissue was homogenized in a glass Teflon homogenizer in synaptic protein extraction reagent (Syn-PER; Thermo Scientific, Rockford, IL, USA) supplemented with a protease and phosphatase inhibitor cocktail (Thermo Scientific). The homogenate was centrifuged at 1,200 × *g* for 10 minutes at 4°C to remove cell debris, and the supernatant was centrifuged at 15,000 × *g* for 20 minutes at 4°C. The supernatant (cytosolic fraction) was removed, and the pellets containing the synaptoneurosomes were resuspended in Syn-PER. The protein concentration of the synaptoneurosome fraction was determined by bicinchoninic acid assay (Thermo Scientific).

2.3.2. Western blot analysis of protein levels

Samples were heat-denatured and equal amounts (30 μg) of samples from each animal were separated with SDS-polyacrylamide gel electrophoresis (5% (w/v) stacking and 8% separating gels, 1.5 mm) before electrophoretic transfer onto a 0.45-μm-pore nitrocellulose membrane (Carl Roth, Karlsruhe, Germany) using a semidry transfer system at 0.8 mA/cm^2. Membranes were first blocked for one hour at room temperature in freshly prepared 5% powdered non-fat milk (Carl Roth) in phosphate-buffered saline, and subsequently incubated overnight with primary antibodies with agitation at 4°C. Primary antibodies were diluted in blocking buffer containing 0.1 % Tween 20 (Carl Roth) as follows: Rabbit-anti-GluA1 (1:3,000; Merck Millipore, Darmstadt, Germany), rabbit-anti-GluA2 (1:1,000; Merck Millipore), rabbit-anti-phospho-Ser845 (1:3,000; Merck Millipore), rabbit-anti-phospho-Ser831 (1:750; Merck Millipore), mouse-anti-β-actin (1:10,000; Abcam, Cambridge, UK), rabbit-anti-β-tubulin (1:50,000; Biolegend, San Diego, CA, USA), mouse-anti-PSD95 (1:1,000; BD Biosciences, Heidelberg, Germany). After several washes in phosphate-buffered saline, membranes were incubated in horseradish peroxidase-conjugated anti-rabbit (1:5,000; Merck Millipore) or anti-mouse antibodies (1:4,000; Biolegend, San Diego, CA, USA) for two hours. Horseradish peroxidase activity was detected using the chemiluminescence reagents provided with the ECL kit (Thermo Scientific). Fluorescence images of the blots were obtained with a FUSION-FX7 imaging system (Vilber Lourmat, Marne La Vallée, France). Integrated (background-subtracted) signal intensity for each antibody band was quantified with ImageJ software, and then normalized with reference to the β-actin band, which was used as a loading control. To assess GluA1 phosphorylation, we first probed blots with anti-phospho-Ser845 or anti-phospho-Ser831 antibodies, stripped them and subsequently probed with anti-GluA1 antibody, which recognizes both phosphorylated and non-phosphorylated GluA1. We then determined the ratio of signal obtained with the Ser845 or Ser831 bands and the GluA1 band. Note that all Western blot data of HFD animals were referenced to values of control animals run at the same time and processed on the same protein gels and resulting Western blots. Therefore, in experiment 1, Western blot results can only be presented as paired data sets, *i.e.*, HF1D and respective control samples, and HF3D and respective control samples.

2.3.3. Analyses of leptin, insulin, free fatty acids and corticosterone

In experiment 1, serum concentrations of leptin, insulin and corticosterone were measured using Enzyme-Linked Immunosorbent Assay (ELISA) kits (Enzo Life Sciences Inc., Farmingdale, NY, USA) according to the manufacturer's instructions. Free fatty acids (FFA) were determined using FFA quantification kit (Abcam, Germany).

2.4. Statistical analysis

All data are expressed as means ± SEM. Analyses were performed with GraphPad Prism (GraphPad Software, San Diego, CA, USA). Energy intake, body weight gain, time spent asleep/awake and blood parameters were analyzed via analyses of variance (ANOVA) and unpaired *t* tests as appropriate. Western blot ratio data derived from paired data sets (see above) did not permit analyses comprising both HFD groups and were analyzed via Mann-Whitney *U* tests. A *P* value of $<.05$ was regarded as statistically significant.

3. Results

3.1. HFD increases energy intake and after three days increases wakefulness

First, we assessed the metabolic effects of high-fat feeding. All three groups of rats in experiment 1 (Control, HF1D and HF3D)

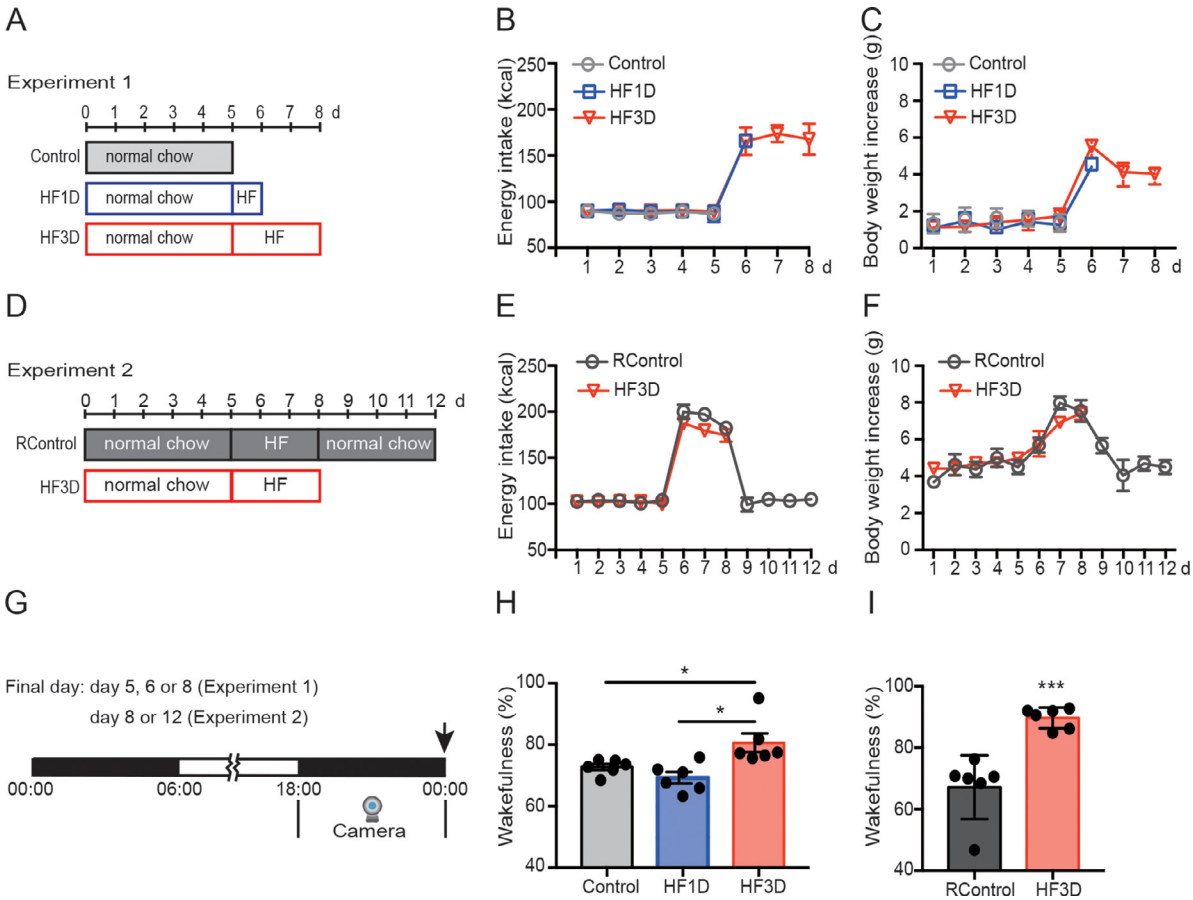

Fig. 1. Study design and results of metabolic and wakefulness/sleep assessments. Experimental timelines of experiments 1 and 2 spanning up to eight and 12 days, respectively. Five days of normal-chow baseline were followed by one or three days of high-fat feeding (A), or subsequent return to normal chow (D), before sacrifice and tissue collection on the last day. B/E, energy intake on experimental days; C/F, mean ± SEM body weight increase per day (difference in body weight to preceding day); G, schedule of the final day of dietary intervention; H/I, mean ± SEM percentage of time spent awake during the final six hours before sacrifice (unpaired *t* tests, * $P<.05$, *** $P<.001$, ns, nonsignificant).

consumed comparable amounts of normal chow during the five baseline days ($P > .14$; Fig. 1B). After the switch to HFD, both high-fat groups increased their energy intake on day 6 compared to that measured in controls on day 5 ($F(2,6) = 17.9$, $P < .01$; $P > .99$ for comparison between HF1D and HF3D), and energy intake in the HF3D group remained elevated during the three days of HFD ($P < .05$ for comparison between days 8 and 5). In accordance with energy intake, body weight increased in both the HF1D and the HF3D groups after the switch to HFD on day 6 ($F(2,15) = 99.5$, $P < .001$; Fig. 1C). A similar pattern was observed in experiment 2. After comparable energy intake during baseline ($P > .28$; Fig. 1E), energy intake increased in both groups during the three days of HFD (each $P < 0.001$); in the recovery control group it returned to baseline levels during the subsequent four recovery days ($P > .74$ for comparison with baseline). Changes in body weight followed those in energy intake, with comparable baseline values ($P > .20$), increases upon HFD ($P < .05$), and subsequent return to baseline levels in the recovery control group ($P > .79$ for comparison between days 8–12 and days 2–4; Fig. 1F).

To investigate the impact of HFD on sleep/wake patterns, we analyzed sleep and wakefulness during the six hours of the dark period before tissue collection (Fig. 1G). One day of HFD compared to normal chow in experiment 1 left time spent awake unaltered ($P = .13$; Fig. 1H). In contrast, three days of HFD generally increased the time spent awake in comparison to controls (experiment 1, $F(2,15) = 7.4$, $P < .01$; Fig. 1H and I). Analyses of relevant hormonal and metabolic blood parameters in experiment 1 corroborated the anabolic impact of high-fat feeding: serum concentrations of insulin, leptin, FFA, and corticosterone were all increased both after one and three days of HFD (all $P < .01$ for ANOVA factor *Group*; Table 1). Insulin and FFA concentrations were higher still after three than one day of HFD. Since animals were freely fed rather than fasted before blood collection, a biasing impact on blood parameters of food intake in these hours cannot be ruled out.

3.2. Three-day high-fat feeding reduces AMPAR levels in the hypothalamus

We hypothesized that even short-term exposure to HFD can trigger global changes in hypothalamic glutamatergic signaling, and investigated the impact of high-fat feeding and associated body weight increases on hypothalamic AMPA receptors by measuring GluA1 and GluA2 subunits and GluA1 phosphorylation in synaptoneurosomes. We found in experiment 1 that three days, but not one day of high-fat feeding compared to normal chow triggered marked reductions in AMPAR levels, *i.e.*, both in GluA1 (Fig. 2A andE) and GluA2 subunits (Fig. 2B and F). In conjunction with the decrease in GluA1, the HF3D group also displayed decreased levels of GluA1 phosphorylated at Ser845 (Fig. 2G; all $P < .05$), while the respective decrease in GluA1 phosphorylation at Ser831 did not

Table 1
Blood parameters in experiment 1.

	Control	HF1D	HF3D
Insulin (ng/mL)	1.18 ± 0.05	1.46 ± 0.05**	1.87 ± 0.01***,###
Leptin (ng/mL)	3.87 ± 0.31	5.44 ± 0.57*	6.34 ± 0.26***
FFA (mEq/L)	0.26 ± 0.04	0.50 ± 0.04**	0.68 ± 0.05***,#
Corticosterone (ng/mL)	261.48 ± 38.60	489.57 ± 61.90*	583.34 ± 48.30***

Serum concentrations of insulin, leptin, free fatty acids (FFA) and corticosterone were determined in rats after one day of high-fat feeding (HF1D) and three days of high-fat feeding (HF3D) and compared to results in rats on normal chow (Control). Values are mean ± SEM ($n = 6$ rats/group); unpaired t tests, $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$ vs. Control; $^{\#}P < .05$, $^{\#\#\#}P < .001$ vs. HF1D.

reach significance ($P = .24$; Fig. 2H; see Fig. 2 C and D for the n.s. results of the one-day intervention). These results were robustly corroborated in experiment 2 in which AMPAR levels were compared between animals after three days of HFD and animals fed the three-day HFD and returned to standard chow for the subsequent four days (recovery control). Here, levels of both GluA1 and GluA2 AMPARs were significantly reduced in the HF3D compared to the recovery control group ($P < .01$; Fig. 2I and J), and the decrease in GluA1 was accompanied by reduced phosphorylation at Ser845 and Ser831 ($P < .05$; Fig. 2K and L). In accordance with our finding of decreases both in total levels of GluA1 and in its phosphorylation at the two sites, we did not observe diet-induced changes in the ratios between GluA1 phosphorylation at Ser845 and, respectively, Ser831 and the respective total GluA1 contents (all $P > .31$; data not shown).

We scrutinized our findings and asked if the observed changes are specific to synaptoneurosomes, or whether high-fat feeding affects the overall cellular levels of AMPARs. To this end, we mea-

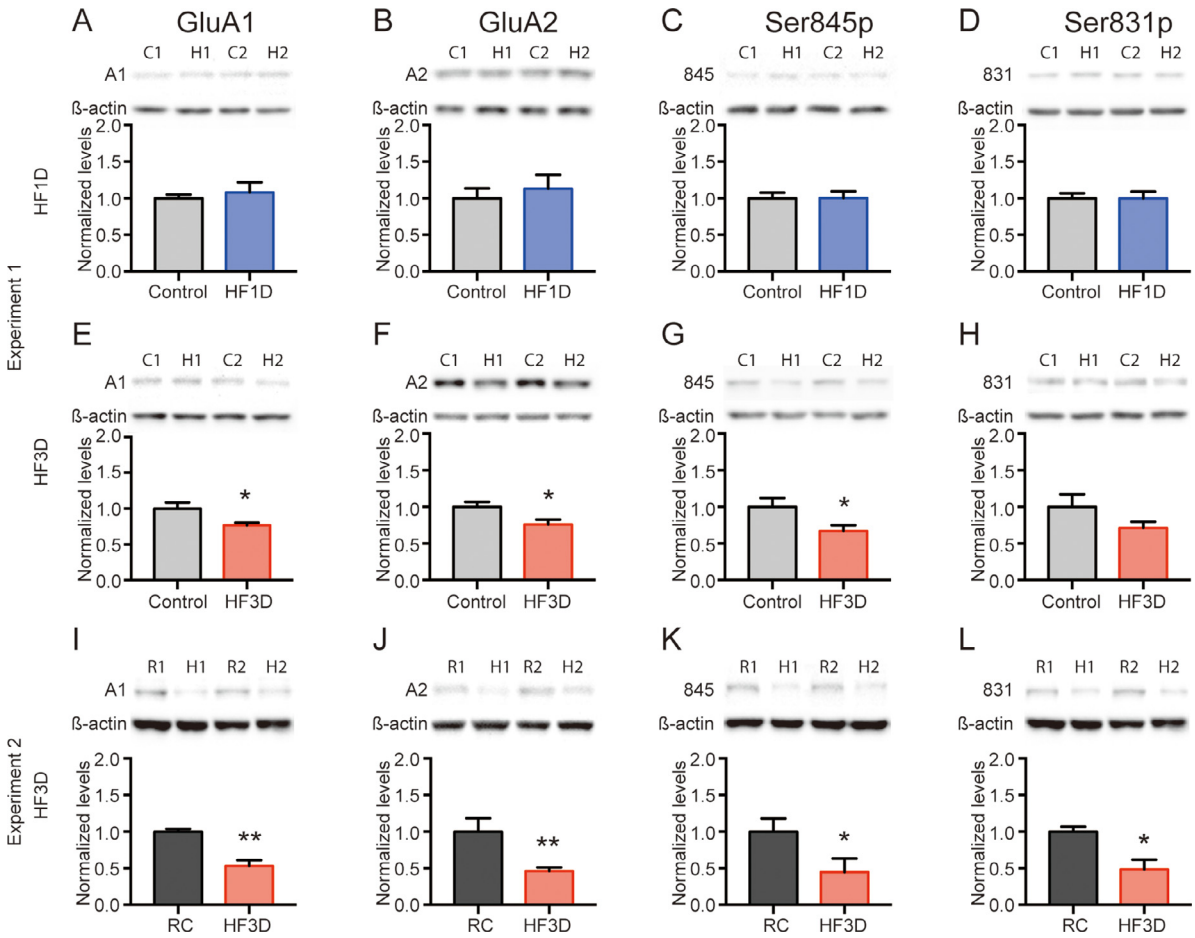

Fig. 2. Suppressive effect of high-fat feeding on AMPARs in hypothalamic synaptoneurosomes. In experiment 1 (A–H), levels of GluA1 and GluA2 subunits of AMPARs and GluA1 phosphorylation at Ser845 and Ser831 in hypothalamic synaptoneurosomes of rats after one day of high-fat feeding (HF1D; A–D) and three days of high-fat feeding (HF3D; E-H) were compared to findings in rats on normal chow (Control). In experiment 2 (I–L), respective measures in animals after three days of high-fat feeding (HF3D) were compared to those obtained in animals after three days of high-fat feeding and four subsequent days of normal-chow feeding (Rcontrol/RC, recovery control). Representative immunoblots (C, Control; H, high-fat feeding; R, recovery control) and quantification of the gels are presented. Values are mean ± SEM (n = 6 rats/group); Mann-Whitney U tests, * $P<.05$, ** $P<.01$.

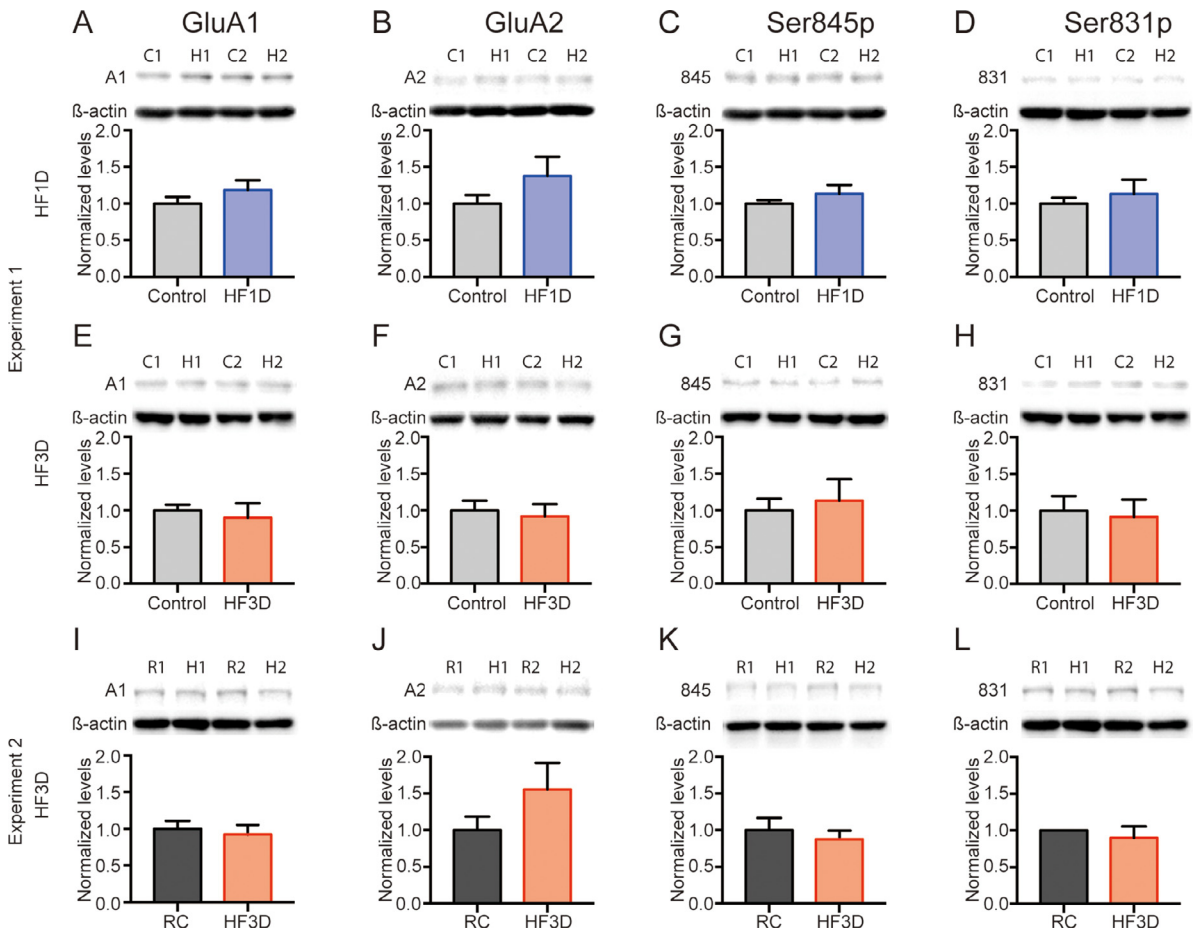

Fig. 3. AMPAR subunit levels in hypothalamic supernatant. In experiment 1 (A–H), levels of GluA1 and GluA2 AMPAR subunits as well as GluA1 phosphorylation at Ser845 and Ser831 in hypothalamic supernatant of rats after one day of high-fat feeding (HF1D; A–D) and three days of high-fat feeding (HF3D; E–H) were compared to findings in rats on normal chow (Control). In experiment 2 (I–L), respective measures in animals after three days of high-fat feeding (HF3D) were compared to results obtained in rats after three days of HFD and four subsequent days of normal-chow feeding (RC, recovery control). Representative immunoblots (C, Control; H, high-fat feeding; R, recovery control) and quantification of the gels are presented. Values are mean ± SEM (n = 6 rats/group); all P>.31 (Mann-Whitney U test).

sured GluA1 and GluA2 content as well as GluA1 phosphorylation in the supernatant fraction of our tissue preparation, which contains mainly the cytosolic components of the neurons. In contrast to our results in synaptoneurosomes, we did not detect any diet-induced changes (all $P > .24$; Fig. 3).

3.3. High-fat feeding does not decrease cortical AMPAR levels

We next turned to assessing markers of synaptic plasticity in the cortex of the animals exposed to high-fat feeding. This structure was chosen as a control region because in previous experiments, neither presynaptic glutamate release nor postsynaptic AMPARs on pyramidal neurons in orbitofrontal cortex changed in response to (albeit long-term) exposure to cafeteria diet [35]. Consequently, we detected a pattern distinct from the decrease in hypothalamic AMPARs: in both experiments, three days of high-fat in comparison to normal-chow feeding appeared to increase rather than decrease levels of cortical GluA1 and GluA2 AMPAR subunits (Fig. 4E and F and I and J) and also the level of GluA1 phosphorylated at Ser845 (Fig. 4G and K) and Ser831 (Fig. 4H and L). Significant or trendwise signs of HF3D-induced increases were restricted to GluA2 ($P < .03$; Fig. 4F) and GluA1 phosphorylation at Ser845 ($P = .06$) in experiment 1. These findings robustly indicate that three-day high-fat feeding does not decrease markers of synaptic plasticity in cortex. Moreover, one day of high-fat feeding did not elicit any changes (all $P > .24$; Fig. 4A–D). In accordance with our findings in the hypothalamus, high-fat feeding neither affected AMPAR subunit levels or GluA1 phosphorylation in the supernatant fraction (all $P > .09$; Fig. 5).

4. Discussion

We demonstrate that high-fat feeding decreases synaptic AMPAR levels in hypothalamus, but not in cortex. This effect develops across the first three days after the transition from regular diet to HFD and is reversible by returning animals to a regular diet. High-fat feeding for three days stimulated energy intake and increased body weight, along with an increase in serum insulin, leptin, FFA, and corticosterone concentrations, as well as prolonged wakefulness. Contrary to our expectations, HFD reduced rather than increased overall levels of hypothalamic AMPARs containing the subunits GluA1 and GluA2 and decreased GluA1 phosphorylation at Ser845 and Ser831, pointing toward a net decrease in overall synaptic input along with a down-regulation of receptor function and/or synaptic stability at synapses. In contrast, cortical levels of GluA1 and GluA2 AMPARs and Ser845p showed a tendency to increase rather than decrease after three days of HFD. In both experiments, changes in AMPARs were absent in supernatant and, therefore, specific to synaptic receptors. Although one day of HFD was sufficient to trigger an immediate increase in energy intake

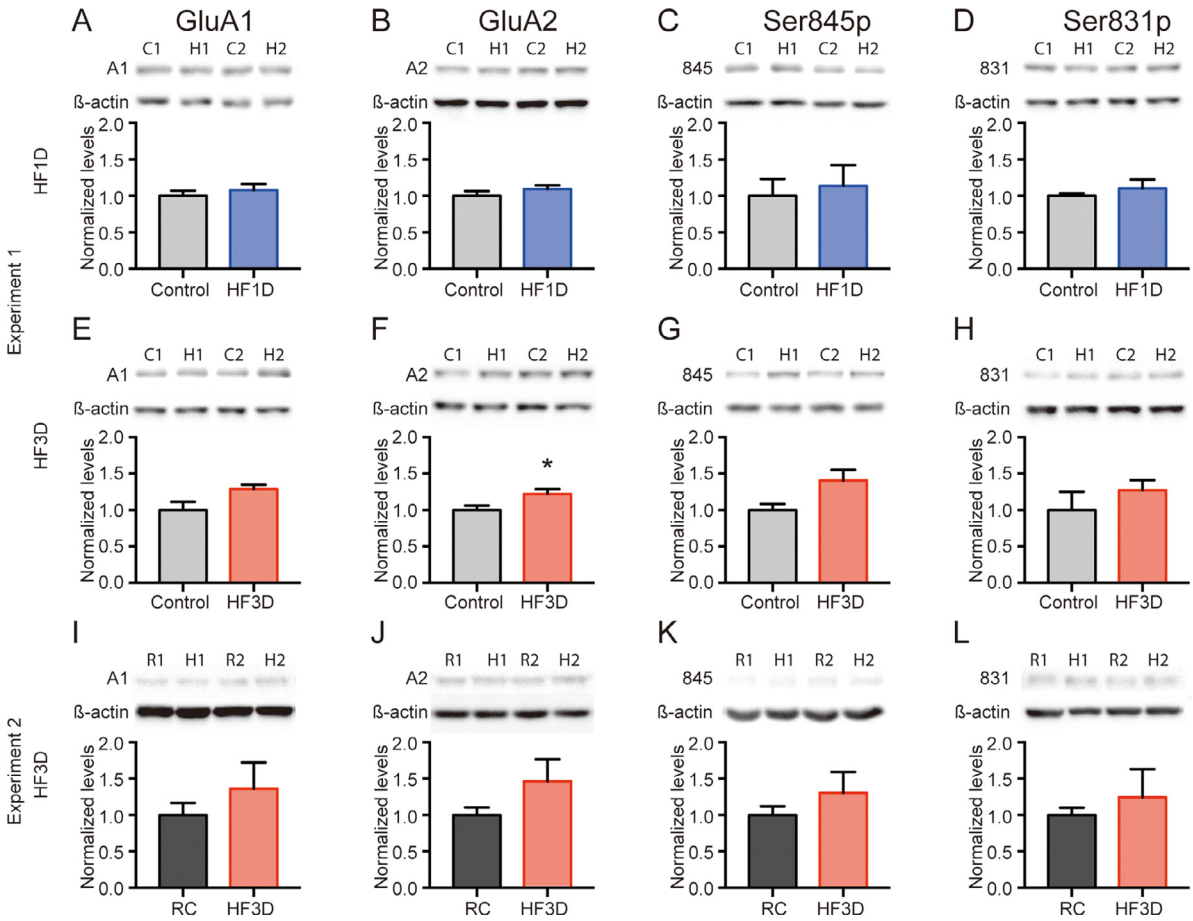

Fig. 4. High-fat feeding does not decrease AMPARs in cortical synaptoneurosomes. In experiment 1 (A–H), levels of GluA1 and GluA2 subunits of AMPARs and GluA1 phosphorylation at Ser845 and Ser831 in left cortical synaptoneurosomes of rats after one day of high-fat feeding (HF1D; A–D) and three days of high-fat feeding (HF3D; E–H) were compared to findings in rats on normal chow (Control). In experiment 2 (I–L), respective measures in animals after three days of high-fat feeding (HF3D) were compared to those obtained in rats after three days of high-fat feeding and four subsequent days of normal-chow feeding (Rcontrol/RC, recovery control). Representative immunoblots (C, Control; H, high-fat feeding; R, recovery control) and quantification of the gels are shown. Values are mean ± SEM (n = 6 rats/group); Mann-Whitney U tests, * P<.05.

and body weight, as well as in related blood markers, in the absence of changes in sleep/wake behavior, this shorter feeding intervention did not alter markers of synaptic plasticity. Changes in the availability of nutrients like FFA in the body periphery and related neuronal and endocrine signals including insulin and leptin are constantly tracked by the brain, in particular hypothalamic ventromedial and arcuate nuclei [23,36]. Against this background, our results indicate a distinct, reversible pattern of net synaptic scaling in association with short-term high-fat feeding [25,26] that precedes the manifestation of obesity and associated synaptic changes [35,37], and may underlie global regulatory efforts to counteract diet-induced body weight gain.

Recent findings in mice indicate that the positive energy balance resulting from 10 days of high-fat feeding leads to hypothalamic expression of calcium-permeable (*i.e.*, GluA2-lacking) AMPARs in POMC neurons via the leptin-mTOR pathway [26]. In related experiments, one day as well as three days of HFD in mice induced an increase in markers of synaptic plasticity in the arcuate but not the paraventricular nucleus and lateral hypothalamus, along with increased glutamatergic input onto POMC neurons after three days of HFD, which were mediated by increased levels of cell-surface glycan polysialic acid molecule (PSA) [25]. Our result of general synaptic down-regulation of AMPARs after three days, but not one day of HFD may reflect a net effect of glutamatergic synaptic scaling in the hypothalamus, and therefore does not exclude concurrent local synaptic up-regulation in specific circuits. The arcuate system harbors orexigenic neurons co-expressing neuropeptide Y, AgRP, and GABA [38] as well as anorexigenic cells expressing POMC-derived peptides including alpha-melanocyte stimulating hormone (alpha-MSH), and cocaine-and-amphetamine-regulated transcript (CART) [22,39] and both populations receive glutamatergic input that is modulated by fasting and/or feeding [40–42]. Moreover, as a central hub of homeostatic autonomic and hormonal control, hypothalamic pathways regulate functions including hypothalamic-pituitary-adrenal stress axis activity, which itself interacts with body weight control [43]. Thus, the observed net synaptic down-regulation, which emerged in conjunction with a strong increase in corticosterone levels, might result from changes that occur in different neuronal networks and might even be of opposite polarity, so that immediate conclusions on its functional significance are not warranted. HFD-induced hypothalamic down-regulation also emerged in comparison to HFD-exposed control animals returned to normal chow. This indicates that the effect (1) is rapidly reversible by diet normalization, (2) is triggered by high-fat food per se rather than the associated elevation in body weight, which in absolute terms was still present after return to normal chow, and (3) does not depend on the novelty of high-fat feeding. In accordance, providing animals with a novel isocaloric control diet remained without effect on arcuate PSA levels in the study by Benani et al. [25].

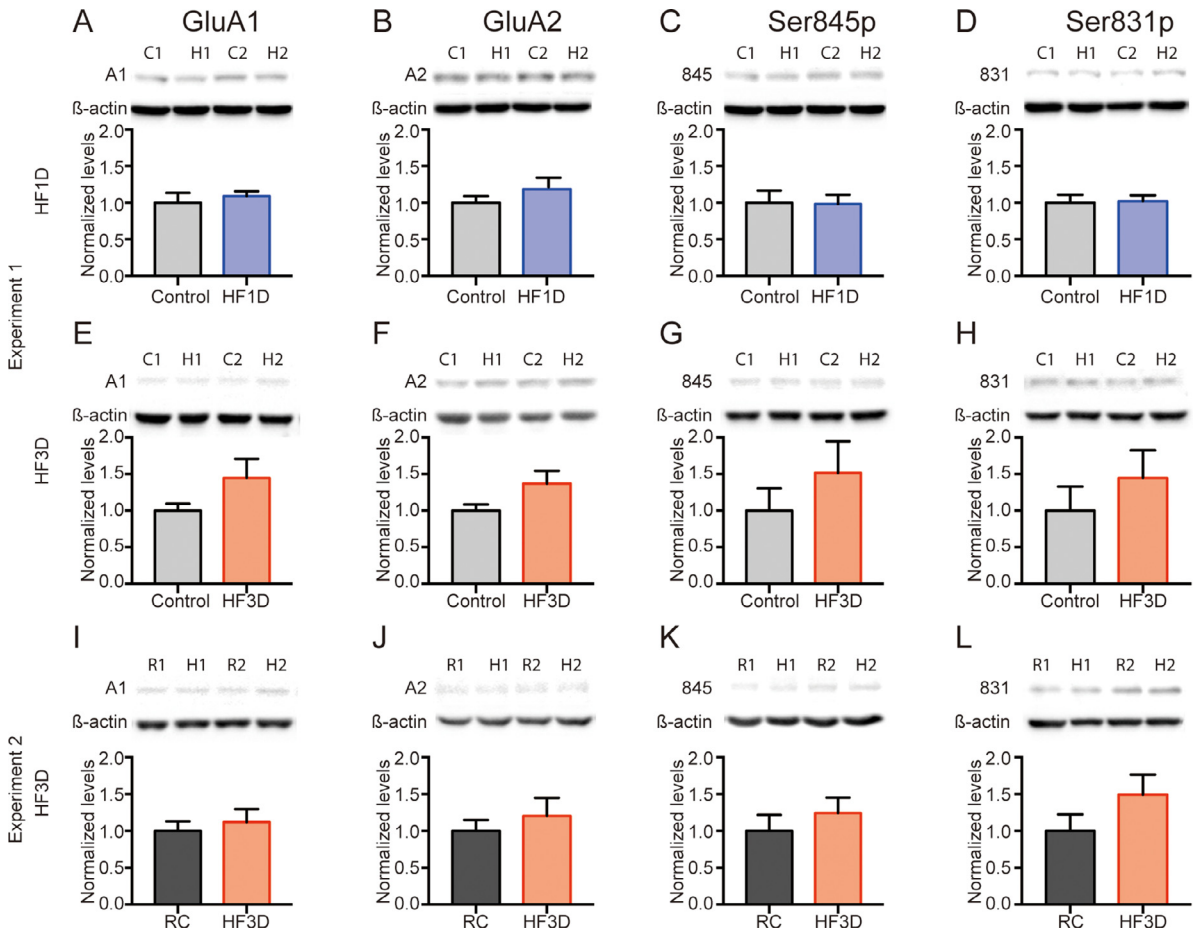

Fig. 5. AMPAR subunit levels in cortical supernatant. In experiment 1 (A-H), levels of GluA1 and GluA2 AMPAR subunits as well as GluA1 phosphorylation at Ser845 and Ser831 in cortical supernatant of rats after one day of high-fat feeding (HF1D; A–D) and three days of high-fat feeding (HF3D; E–H) were compared to findings in rats on normal chow (Control). In experiment 2 (I–L), respective measures in animals after three days of high-fat feeding (HF3D) were compared to results obtained in rats after three days of HFD and four subsequent days of normal-chow feeding (RC, recovery control). Representative immunoblots (C, Control; H, high-fat feeding; R, recovery control) and quantification of the gels are shown. Values are mean ± SEM ($n = 6$ rats/group); all P>.09 (Mann-Whitney U test).

We also found that in cortex, in contrast to hypothalamus, high-fat feeding in general did not affect or rather even increased AMPAR levels. Prolonged high-fat feeding and obesity are associated with decreased activation in dorsolateral and anterior prefrontal cortices [44] and decreased spine density in the lateral orbitofrontal cortex [35], and detrimental effects of HFD and obesity on cognitive function are generally well-known [8, 45–47]. While previous research on non-hypothalamic effects of dietary changes on AMPAR levels and function focused on the brain's reward circuitry [37,48], the pattern observed in our study suggests that the short-term global synaptic impact of high-fat feeding in the cortex is limited, and might even imply an acute compensatory response of superordinate brain centers in the early stages of high-fat feeding. Localized changes in cortical networks were not investigated in the present study. It is also to note that our experiments, like most related studies [e.g., 1,25,27,35,37], were performed in males; considering previous reports of sexually dimorphic effects of environmental changes on synaptic plasticity [49,50], the impact of high-fat feeding on hypothalamic and cortical AMPAR levels in females is in need of clarification.

Animals on HFD spent more time awake than control animals in the final six hours of three-, but not one-day HFD. Unchanged wakefulness during one day of high-fat feeding argues against the assumption that novelty- or palatability-induced increases in foraging acted as a wake-promoting factor in the HF3D group. Longer periods of high-calorie feeding in rodents have been repeatedly, although not unanimously [51] observed to decrease wakefulness [52–54], possibly via increases in sleep-promoting cytokines like tumor necrosis factor and interleukin-6 [55,56]. In humans, increased fat and sugar intake for one day yielded signs of lighter sleep [57], and fat intake was observed to be associated with decreased sleep duration [58]. In conjunction with these findings, our results suggest that initial HFD-associated increases in wakefulness give way to more pronounced sleep once overweight and related sleep-regulatory changes become manifest. Wakefulness and sleep are associated with net increases and, respectively, decreases in synaptic strength in cortex and hippocampus as reflected by changes in synaptic AMPAR GluA1 and GluA2 subunits and GluA1 phosphorylation at Ser845 and Ser831 [27,59], observations that support the "synaptic homeostasis hypothesis" of net synaptic potentiation during wakefulness and depression during sleep [28]. It is, therefore, tempting to speculate that the HFD diet-associated increases in wakefulness observed here contributed to signs of cortical synaptic up-regulation.

In sum, our findings indicate that short-term high-fat feeding in rats down-regulates hypothalamic but not cortical synaptic strength, and that this effect is rapidly reversible. Although our results do not allow insights into the rewiring of specific neu-

ronal populations that fine-tune food intake control, they suggest that global scaling processes accompany the initial phase of high-calorie intake to shift the overall hypothalamic activity balance and, possibly, to counteract anabolic drive and weight gain. It will be important to investigate how long-term exposure to high-calorie food eventually tips these synaptic scales, and how they can be recalibrated to promote energy homeostasis.

Author contributions

Jianfeng Liu: Conceptualization, Methodology, Investigation, Formal analysis, Writing – Original draft preparation, Visualization. **Stoyan Dimitrov:** Conceptualization, Methodology, Investigation, Validation, Writing – Original draft preparation. **Anuck Sawangjit:** Methodology, Investigation. **Jan Born:** Conceptualization, Validation. **Ingrid Ehrlich:** Conceptualization, Methodology, Validation, Writing – Original draft preparation, Writing – Review & Editing, Supervision. **Manfred Hallschmid:** Conceptualization, Validation, Writing – Original draft preparation, Writing – Review & Editing, Supervision, Project administration.

Declaration of competing interests

The authors declare no conflicts of interest.

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