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ORIGINAL ARTICLE

Hypervulnerability of the adolescent prefrontal cortex to nutritional stress via reelin deficiency

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Overconsumption of high-fat diets (HFDs) can critically affect synaptic and cognitive functions within telencephalic structures such as the medial prefrontal cortex (mPFC). The underlying mechanisms, however, remain largely unknown. Here we show that adolescence is a sensitive period for the emergence of prefrontal cognitive deficits in response to HFD. We establish that the synaptic modulator reelin (RELN) is a critical mediator of this vulnerability because (1) periadolescent HFD (pHFD) selectively downregulates prefrontal RELN$^+$ cells and (2) augmenting mPFC RELN levels using transgenesis or prefrontal pharmacology prevents the pHFD-induced prefrontal cognitive deficits. We further identify *N*-methyl-D-aspartate-dependent long-term depression (NMDA-LTD) at prefrontal excitatory synapses as a synaptic signature of this association because pHFD abolishes NMDA-LTD, a function that is restored by RELN overexpression. We believe this study provides the first mechanistic insight into the vulnerability of the adolescent mPFC towards nutritional stress, such as HFDs. Our findings have primary relevance to obese individuals who are at an increased risk of developing neurological cognitive comorbidities, and may extend to multiple neuropsychiatric and neurological disorders in which RELN deficiency is a common feature.

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INTRODUCTION

The quality of the human diet has undergone rapid and profound changes in the past century. Within a few decades, there has been a radical shift from an agriculture-based dietary regimen towards one composed of high-calorie processed foods strongly enriched in their levels of saturated fatty acids.[1,2] Some of these dietary customs are insidious as they have become a generalized phenomenon in western countries,[3] making their negative health effects appear seemingly harmless.[4] Chronic intake of high-fat diets (HFDs) is recognized by many researchers as representing one of the primary risk factors in the development of obesity and associated metabolic anomalies.[5] Although much less appreciated thus far, excessive consumption of HFD can also represent a form of nutritional stress for the brain whereby it can lead to neuronal adaptations and impair various cognitive functions.[6] This emerging topic is of great concern for mental health as HFD may increase the prevalence and/or severity of mental disorders harboring dysfunctions in the cognitive domains.[6–8] In this context, dietary effects on the hippocampus have received the widest appreciation,[9–11] partly because chronic HFD intake is a recognized risk factor for Alzheimer's disease (AD) and AD-associated impairments in hippocampal function.[8]

According to human investigations, however, the cognitive effects of HFD are not limited to hippocampal-regulated functions, but rather extend to other brain regions, in particular the medial prefrontal cortex (mPFC).[12,13] For example, in healthy young adult males, a short-term HFD treatment (75% of energy) is sufficient to reduce prefrontal-related attentional functions and speed of retrieval in executive tasks.[12,13] Causality for such associations has also been established in two adult rat models of obesity showing that chronic HFD exposure can elicit impairments in cognitive functions that rely on the mPFC.[14,15] It remains to be established, however, what molecular mechanisms mediate the pathological link between excessive HFD consumption and cognitive dysfunction, and how these mechanisms may specifically affect certain brain regions at relevant vulnerable time periods.

One distinctive feature of the mPFC is its protracted maturation, which, unlike in the rest of the brain, is actively sustained throughout adolescence until early adulthood. Thus, the mPFC is widely recognized as being the last brain region to reach full maturity in humans[16] and rodent models.[17] Although this protracted maturation is thought to confer an extended period of plasticity that supports experience-dependent learning, it also provides a neural basis for developmental disruption by periadolescent environmental insults,[18–20] such as HFD. In this respect,

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several human studies have shown that obesity in general, and consumption of fat-rich diets in particular, are associated with poorer cognitive functions, reduced executive performance and volumetric changes in frontal cortical regions in adolescent individuals.[21–26] Accordingly, it has been hypothesized that the relative immaturity of the mPFC in adolescent subjects may render this brain region particularly vulnerable towards the deleterious effects of HFD and/or obesity.[21]

This pathological link may be particularly relevant to the adolescent population for various reasons. First, adolescence represents a time of amplified caloric needs and increased appetite.[17,27] Second, it is the time of first independence, during which food choices are more frequently made by adolescents, rather than their parents, with an important influence of media and peer pressure favoring less healthy nutritional choices.[17,27] Therefore, in addition to being a time of active mPFC maturation, adolescence also represents a highly vulnerable period in terms of nutritional decisions.

Here we investigate nutritionally induced periods of vulnerability using a mouse model of chronic HFD exposure, one of the most commonly used and etiologically valid model system in obesity research.[5] Our first aim was to determine whether adolescence represents a period of increased vulnerability towards the emergence of prefrontal cognitive impairments elicited by HFD, and second, to identify the mechanistic basis of this vulnerability. We focused our investigations on the reelin (RELN) protein, which is known to be critically involved in the regulation of prefrontal cortical activity.[28–31] Recent findings have suggested that perturbations in excitatory neurotransmission within the mPFC are implicated in the etiology of HFD cognitive impairments,[14] and such functions are known to be directly regulated by the RELN protein.[29,31–35] Importantly, RELN is a sensitive target of early-life environmental insults[36–38] and was proposed to represent a marker of resilience that exerts protective effects against cellular and environmental stressors,[39–42] thus making it a primary candidate that might drive prefrontal vulnerability.

MATERIALS AND METHODS

Animals and dietary manipulations

We used C57BL/6 N or transgenic male mice overexpressing RELN under the control of the calcium–calmodulin-dependent kinase IIα promoter (as in our previous studies[39,41,43]). Adolescent and adult cohorts had access to HFD (63% calories from fat; KLIBA, Kaiseraugst, Switzerland) or to a regular control diet (CD; KLIBA) from postnatal day (P) 28 or 70 onwards, respectively. All procedures were approved by the Zurich Cantonal Veterinary Office or by the European Union (approval no. B 13-055-19).

Behavioral testing

As further outlined below, spatial working memory was examined using a novelty recognition test in the Y-maze or a matching-to-position paradigm in the Morris water maze; cognitive flexibility was assessed using a left-right discrimination reversal learning test in the water maze; fear conditioning and extinction were measured using cued fear conditioning tests and sensorimotor gating was examined using the paradigm of prepulse inhibition (PPI) of the acoustic startle reflex.

Western blots, immunohistochemistry and unbiased stereological estimations

The following primary antibodies were used for western blots: rabbit anti-phosphorylated-disabled-1 (p-Dab1) (3327; 1:500; Cell Signaling, Allschwil, Switzerland) and rabbit anti-Dab1 (ab5840; 1:1000; Millipore, Darmstadt, Germany); and for immunohistochemistry: mouse-G10 anti-RELN (MAB5364; 1:2000; Millipore, Temecula, CA, USA), rabbit anti-parvalbumin (PV) (PV27; 1:10 000; Swant, Marly, Switzerland) and mouse anti-glutamate decarboxylase 67 (GAD_{67}) (MAB5406; 1:1000; Millipore, USA). Unbiased stereological estimations were determined blind to the treatment conditions using the optical fractionator method and Stereo Investigator software (version 6.50.1; MicroBrightField, Delft, The Netherlands) and confirmed in two independent counting sessions.

Electrophysiology

All recordings were carried out in the prelimbic mPFC layer V/VI. Extracellular field excitatory postsynaptic potentials (fEPSPs) were evoked at 0.1 Hz in layers II/III[29] in the presence of picrotoxin (Sigma, St. Quentin Fallavier, France). Long-term depression (LTD) was induced by a 15 min stimulation at 1 Hz. The magnitude of LTD was calculated 30–35 min afterstimulation as percentage baseline. Spontaneous excitatory postsynaptic currents (sEPSCs) were recorded in whole-cell voltage-clamp configuration in layer V/VI pyramidal neurons. AMPA receptor-mediated sEPSCs (AMPAR-sEPSCs) were recorded at −70 mV and *N*-methyl-D-aspartate receptor-sEPSCs (NMDAR-sEPSCs) were recorded at +40 mV in the presence of 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[*f*]quinoxaline-2,3-dione (NBQX) (20 μM). Current–voltage (*I–V*) curves testing pyramidal neuron intrinsic properties were obtained by applying a series of hyperpolarizing and depolarizing current steps immediately after breaking into the cell.[44]

Intra-mPFC rRELN administration

In a first cohort of mice, recombinant RELN (rRELN) (0.5 μg μl^{-1}; 0.75 μl; no. 3820-MR-025; R&D Systems, Abingdon, UK), which was validated and used in previous studies,[45] or vehicle (0.1% bovine serum albumin in phosphate-buffered saline) solution was directly injected into the mPFC bilaterally (anteroposterior: +1.85 mm; medial–lateral: ±0.50 mm; dorsal–ventral: −3.00 mm) using glass pipettes; pipettes were left in place for 4 min after injection. In a second cohort, rRELN or vehicle were injected through an injector cannula using a microsyringe pump controller and Hamilton syringe at an average rate of 1μl min^{-1} through cannulas previously positioned in the mPFC (anteroposterior: +1.85 mm; medial–lateral: +0.50 mm; dorsal–ventral: −2.00 mm). Injection cannulas were left in place for 1 min after injection. Mice were re-exposed to the discrimination learning task acquired previously until reaching criterion and then tested in a reversal task. Testing occurred days 3 to 9 after rRELN/vehicle injections (first reversal day on day 4) in accordance with previous protocols.[46] Locomotor activity was assessed under the same conditions.

Statistical analyses

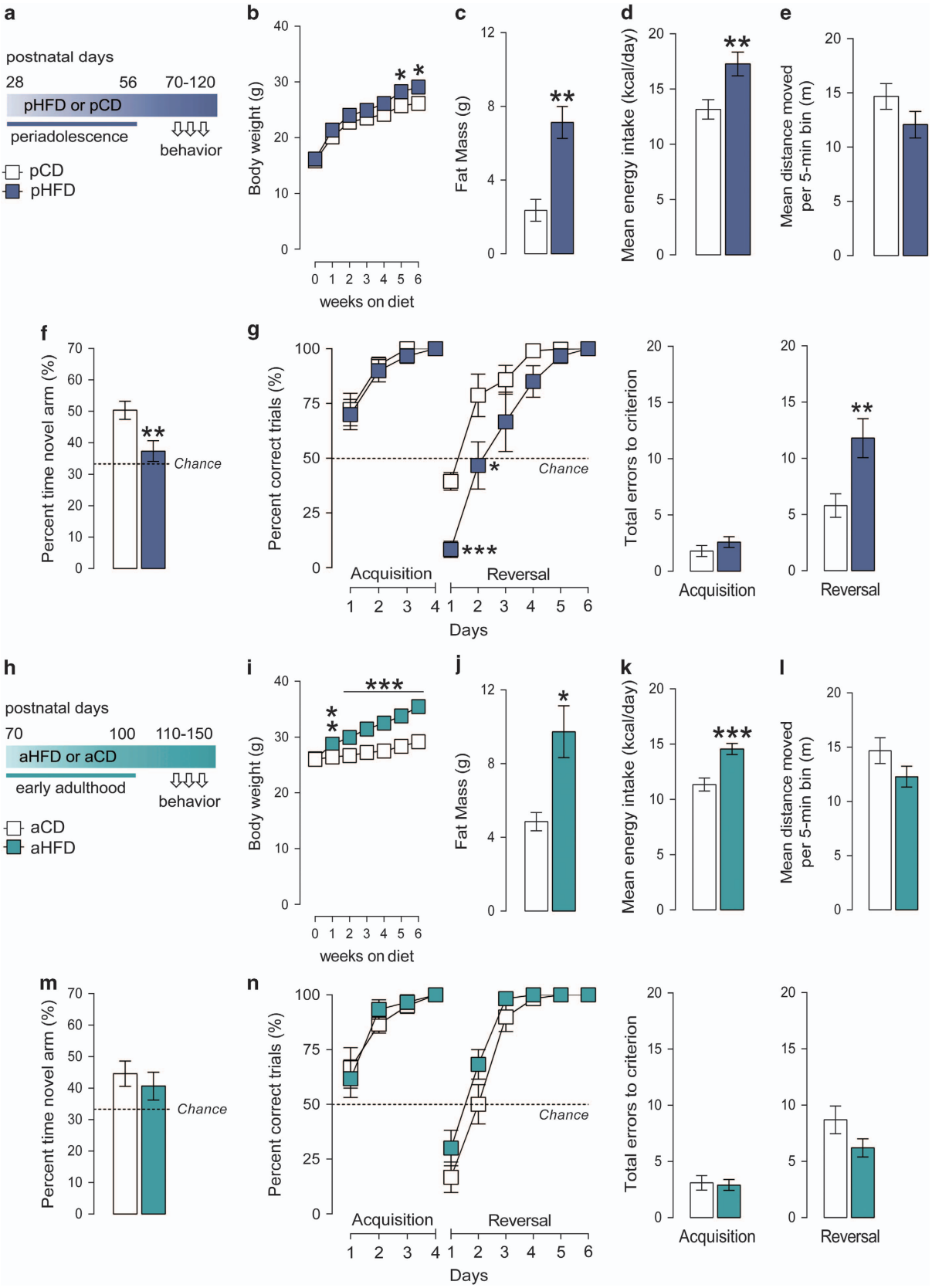
All data were analyzed with GraphPad Prism (version 6.0, La Jolla, CA, USA) or StatView (version 5.0, SAS Institute, Wallisellen, Switzerland) using an unpaired Student's *t*-test (two-tailed), parametric analysis of variance followed by Fisher's least significant difference *post hoc* comparisons whenever appropriate or nonparametric tests (Mann-Whitney and Kruskal-Wallis tests) when the data did not meet the criteria of normal distribution or equality of variance. Statistical significance was $P < 0.05$. Specific statistical methods and outcomes are outlined in Supplementary Tables S1–S5.

RESULTS

Adolescents are prone to developing mPFC-dependent cognitive impairments following exposure to HFD

To compare the cognitive effects of nutritional stress during adolescence and adulthood, we exposed mice to HFDs or CDs during periadolescent (pHFD and pCD) or adult (aHFD and aCD) periods (Figures 1a and h). As expected,[5] both pHFD and aHFD treatments led to hyperphagia and obesity compared with their age-matched controls (Figures 1b–d and i–k). We then tested the same mice on several cognitive tests which depend on mPFC function.[47,48] We first observed that pHFD mice displayed impaired working memory in a Y-maze spatial recognition test. In this test, pCD mice showed an ~50% preference for a previously unexplored arm of a familiar Y-maze (chance level at 33.3%), whereas this preference was fully blunted in pHFD animals (Figure 1f). Working memory deficits in pHFD animals were further confirmed in a matched-to-position paradigm in the Morris water maze (Supplementary Figure S1). In addition, pHFD mice demonstrated deficits in cognitive flexibility, which is also known to be regulated by the mPFC.[48] Whereas pHFD mice did not differ from pCD controls during the initial acquisition of a left-right discrimination task, they displayed a selective delay in learning the reversed response-outcome contingencies (Figure 1g). The mPFC-related cognitive deficits induced by pHFD were not confounded by

changes in general locomotor activity as assessed in an open-field exploration test (Figure 1e). In stark contrast to pHFD animals, mice exposed to the same HFD regimen in adulthood (i.e., aHFD mice) gained more weight than pHFD mice (Figures 1b and i) but did not display similar mPFC-related functional impairments (Figures 1m and n) when compared with their age-matched



controls (aCD). Taken together, these findings demonstrate that adolescent subjects are more vulnerable than adults towards the development of prefrontal cognitive deficits following chronic HFD treatment.

pHFD leads to a cell-, region- and time-specific reduction in RELN$^+$ cells

We hypothesized that the RELN protein could be a candidate factor shaping the differential vulnerability of adolescents and adults towards HFD-induced prefrontal deficits. In postnatal life, RELN is primarily expressed by a subgroup of GABA (γ-aminobutyric acid) interneurons[49] and assumes key roles in the regulation of excitatory neurotransmission, synaptic plasticity and cognition.[29,33–35] The RELN protein is known to be a sensitive target of early-life environmental insults,[36–38] and at the same time, it can convey resilience against the deleterious effects of stress.[42] Indeed, in adult animals, RELN does not seem to overtly affect behavioral and cognitive functions under baseline conditions,[30,40,50,51] but instead is thought to act as a protective factor that increases the capacity to cope with cellular or environmental stressors.[39–41]

Using immunohistochemical techniques coupled with unbiased stereology after 13 weeks on HFD (P120) (Figure 2a), we found

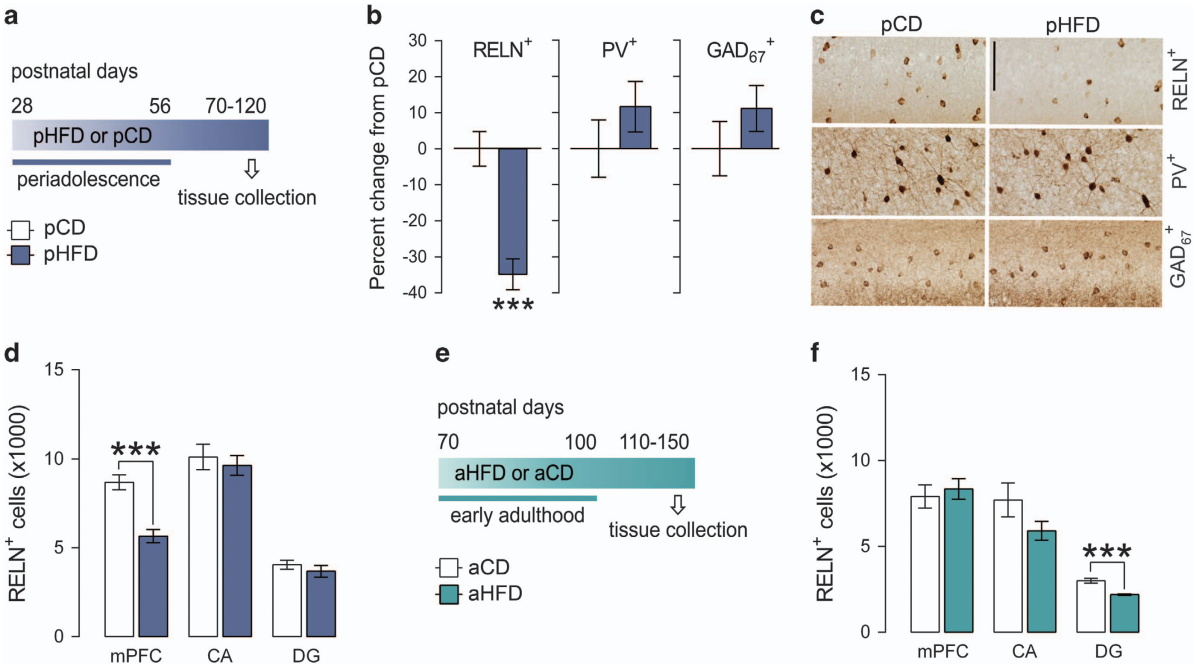


Figure 2. Periadolescent high-fat diet (pHFD) leads to a cell-, region- and time-specific reduction in reelin (RELN)$^+$ cells. (**a**) Experimental design used to assess the neuronal effects of pHFD vs control diet (pCD) starting on postnatal day 28 (P28). (**b**) pHFD led to a selective reduction in the number of medial prefrontal cortex (mPFC) RELN$^+$ cells (5.7 ± 0.4 cells $\times 1000$) as compared with pCD (8.7 ± 0.4 cells $\times 1000$), without affecting parvalbumin (PV)$^+$ and glutamate decarboxylase 67 (GAD_{67})$^+$ cell numbers. The bar plot depicts the percent change in pHFD mice relative to pCD mice. See also Supplementary Figure S2. (**c**) Representative images of RELN-, PV- and GAD_{67}-immunoreactive cells in the mPFC of pCD and pHFD mice. Scale bar = 100 μm. (**d**) pHFD led to a selective reduction in mPFC RELN$^+$ cells, without affecting RELN$^+$ cell numbers in the CA1–CA3 (CA) and dentate gyrus (DG) regions of the hippocampus as compared with pCD. mPFC RELN$^+$ cell estimations are the same data as in (**b**). (**e**) Experimental design used to assess the neuronal effects of adult HFD (aHFD) vs control diet (aCD) starting on P70. (**f**) aHFD did not affect mPFC or CA RELN$^+$ cells as compared with aCD, but led to a reduction of RELN$^+$ cells in the DG. ***$P < 0.001$. All data are means ± s.e.m.

Figure 1. Adolescents are prone to developing medial prefrontal cortex (mPFC)-dependent cognitive impairments following exposure to high-fat diet (HFD). (**a**) Experimental design used to assess the metabolic and cognitive effects of periadolescent HFD (pHFD) vs control diet (pCD) starting on postnatal day 28 (P28). (**b**) pHFD increased body weights as compared with pCD. (**c**) pHFD increased body adiposity as compared with pCD. (**d**) pHFD led to an increase in mean energy intake as compared with pCD. (**e**) pHFD did not affect locomotor activity in an open-field arena as compared with pCD (mean distance moved per 5-min bins). (**f**) pHFD led to a deficit in working memory as indexed by the reduced percent time spent in the novel arm during a Y-maze spatial recognition test in pHFD mice (37.4 ± 3.3% time novel arm) compared with pCD mice (50.4 ± 2.9%). See also Supplementary Figure S1. (**g**) pHFD led to a reduction in discrimination reversal learning as compared with pCD. Line plot: Percent correct trials during the acquisition and reversal of left-right discrimination in a water T-maze. Bar plot: Total number of errors to criterion during the two phases. pHFD and pCD animals required 11.8 ± 1.7 and 5.8 ± 1.1 reversal trials to reach reversal criterion, respectively. (**h**) Experimental design used to assess the metabolic and cognitive effects of adult HFD (aHFD) vs control diet (aCD) starting on P70. (**i**) aHFD increased body weights as compared with aCD. (**j**) aHFD increased body adiposity as compared with aCD. (**k**) aHFD increased mean energy intake as compared with aCD. (**l**) aHFD did not affect locomotor activity in an open-field arena as compared with aCD (mean distance moved per 5-min bins). (**m**) aHFD did not affect working memory as compared with aCD as indexed by the percent time spent in the novel arm during a Y-maze spatial recognition test. (**n**) aHFD did not affect discrimination reversal learning as compared with aCD. Line plot: Percent correct trials during the acquisition and reversal of left-right discrimination in a water T-maze. Bar plot: Total number of errors to criterion during the two phases. The dashed lines represent the performance chance levels in the respective tests. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$: main effects or *post hoc* effects at individual time points. All data are means ± s.e.m.

that pHFD mice displayed an ~35% reduction in prefrontal $RELN^+$ cells as compared with pCD mice (Figures 2b and c). This reduction was evident across all mPFC subregions (Supplementary Figure S2a), but not in the orbitofrontal cortex (Supplementary Figure S2b and Supplementary Discussion), and manifested as an early event emerging at the end of adolescence (P56) (Supplementary Figure S2c). We next examined whether these cellular deficits would extend to other interneuron populations such as PV fast-spiking interneurons, which have previously been implicated in the regulation of prefrontal cognitive functions.[52] The pHFD exposure, however, did not change the density of PV^+ neurons in the mPFC (Figures 2b and c), nor did it modulate the density of the entire GABAergic cell population expressing GAD_{67} (Figures 2b and c). Furthermore, pHFD did not affect RELN levels in the CA1–CA3 (CA) and dentate gyrus (DG) regions of the hippocampus (Figure 2d), indicating a selective pHFD-induced RELN deficit in the mPFC. Finally, this phenotype was specific to pHFD treatments because mPFC RELN levels remained unaffected when the dietary manipulation was restricted to adulthood (Figures 2e and f). Adult HFD did, however, reduce $RELN^+$ cell numbers in the DG (Figure 2f), thus confirming previous findings in adult HFD-exposed animals.[9,11] Altogether, these results demonstrate that excessive intake of HFD during adolescence selectively affects $RELN^+$ cells in a cell-, time- and region-specific manner. The latter findings indicate that RELN deficiency in the mPFC might contribute to the modulation of prefrontal functions following adolescent HFD consumption.

pHFD impairs AMPA- and NMDA-related synaptic functions

We next sought to identify a prefrontal neuronal signature of RELN deficits at the synaptic level.[29,31] Upon secretion and transport through the extracellular space, RELN reaches excitatory synapses on pyramidal neurons,[53,54] where it primarily binds to two lipoprotein receptors expressed in postsynaptic densities, namely very-low-density lipoprotein receptor (VLDLR) and apolipoprotein 2 (ApoER2). Activation of VLDLR and ApoER2 promotes Dab1 phosphorylation, which in turn leads to the activation of signaling pathways that modulate NMDA-dependent synaptic plasticity.[33,34,46,54] Synaptic plasticity is thought to represent one of the main cellular mechanisms for learning and memory processes.[55] Interestingly, cognitive flexibility and working memory have both been linked to NMDA-dependent LTD,[55–57] which in turn might thus provide a cellular mechanism linking deficits in the cognitive domains to adolescent HFD intake.

We found that exposure to pHFD did not affect the expression levels of VLDLR, ApoER2 and Dab1 (Supplementary Figure S3), suggesting that immediate downstream signaling partners of RELN are not affected in pHFD mice. We then examined whether pHFD would lead to an impairment in NMDA-dependent LTD functions, which are known to be regulated by RELN.[34,46] We revealed that in pCD mice, low-frequency stimulation (LFS) of mPFC slices induced a robust LTD (24% depression) of fEPSPs (Figures 3a and b). This effect was blocked by the NMDA antagonist (2*R*)-amino-5-phosphonopentanoate (D-APV), confirming its NMDA nature (Figure 3c). In marked contrast to pCD animals, LTD was fully absent in mice exposed to pHFD treatments (Figure 3b).

NMDA-LTD relies on intact glutamatergic transmission at both NMDARs and AMPARs. It is first triggered by the entry of Ca^{2+} through postsynaptic NMDARs and activation of downstream pathways, after which it critically depends on the internalization of AMPARs at the postsynapse and concomitant reductions in AMPA neurotransmission.[55] Previous studies have shown that RELN affects both NMDA and AMPA synaptic activity via partly independent mechanisms.[28,32,33,35] We therefore recorded AMPAR- and NMDAR-sEPSCs in mPFC pyramidal neurons[29] (Figure 3a). We found that pHFD led to a 24% reduction in the amplitude of AMPAR-sEPSCs when compared with pCD treatments. This effect emerged in the absence of concomitant changes in AMPAR-sEPSC frequency (Figures 3d and e), suggesting that impairments in AMPAR-sEPSCs primarily involved postsynaptic mechanisms.[33,35] We then examined possible changes in NMDAR-sEPSCs following pHFD but did not, however, detect any differences in either charge transfer or frequency (Figures 3d and f).

We next wondered whether pHFD would induce wider functional consequences in deep layer pyramidal neurons in particular with respect to their intrinsic properties (Supplementary Figure S4a). Using current-clamp recordings, we found that input–output relationships were similar in pCD and pHFD mice (Supplementary Figure S4b), as was the resting membrane potential (-76.0 ± 0.9 vs -74.5 ± 0.9 mV). The number of action potentials elicited by a series of somatic positive current steps (Supplementary Figure S4c) and the minimal current necessary to evoke action potential firing (or rheobase) (Supplementary Figure S4d) were also left unchanged by the pHFD treatment. These data thus show that pHFD does not induce broad functional disturbances at prefrontal excitatory synapses. Rather, pHFD elicits a specific perturbation of NMDA-LTD functions, which is likely to arise from impaired RELN signaling and its downstream consequences on AMPA neurotransmission.[34,46,55]

In a last step, we thus aimed at determining whether augmenting RELN levels could restore some of the physiological defects of pHFD mice. We used a model of forebrain-specific RELN overexpression (RELN-OE) that had been established and used in our previous studies (Supplementary Figure S5).[39,41,43] In this model, the RELN transgene is conditionally expressed under the control of the calcium–calmodulin-dependent kinase IIα promoter, thus driving transgene expression exclusively in the postnatal and adult forebrain.[43] We first confirmed that pHFD-fed animals of the control genotype (CON/pHFD) showed similar deficits in prefrontal NMDA-LTD (Figure 3h) as seen previously in C57BL/6N pHFD mice (Figure 3b). We then revealed that overexpression of the RELN protein in pHFD mice (RELN-OE/pHFD) fully prevented this physiological deficit, as illustrated by the presence of normal NMDA-LTD (22% depression) in these animals (Figure 3h).

Altogether, our findings thus identify a prefrontal synaptic signature of RELN deficiency in pHFD mice in the form of an abolished NMDA-LTD and thus strongly support a critical role for the RELN protein in the emergence of prefrontal pathology following adolescent HFD exposure.

Transgenic RELN overexpression prevents prefrontal cognitive dysfunctions induced by pHFD

In a last series of experiments, we explored whether the pHFD-induced cognitive abnormalities could also be prevented by increasing RELN levels. We used two distinct methods to test this hypothesis. The first approach made use of the RELN-OE transgenic model used previously (see Figure 3h). In agreement with previous work,[41] RELN-OE *per se* did not affect basal cognitive functions in control-fed animals (CON/pCD vs RELN-OE/pCD comparisons in Figures 4f–i). Furthermore, RELN-OE did not influence body weight gain, body adiposity changes, energy intake and basal locomotor activity in neither dietary treatment groups (Figures 4b–e), indicating that these variables did not represent confounders in any of the cognitive tests. RELN-OE did, however, fully prevent the pHFD-induced deficits in working memory as assessed in the Y-maze spatial recognition test (Figure 4f). RELN-OE also prevented the pHFD-induced emergence of cognitive inflexibility as assessed using a discrimination reversal learning task (Figure 4g).

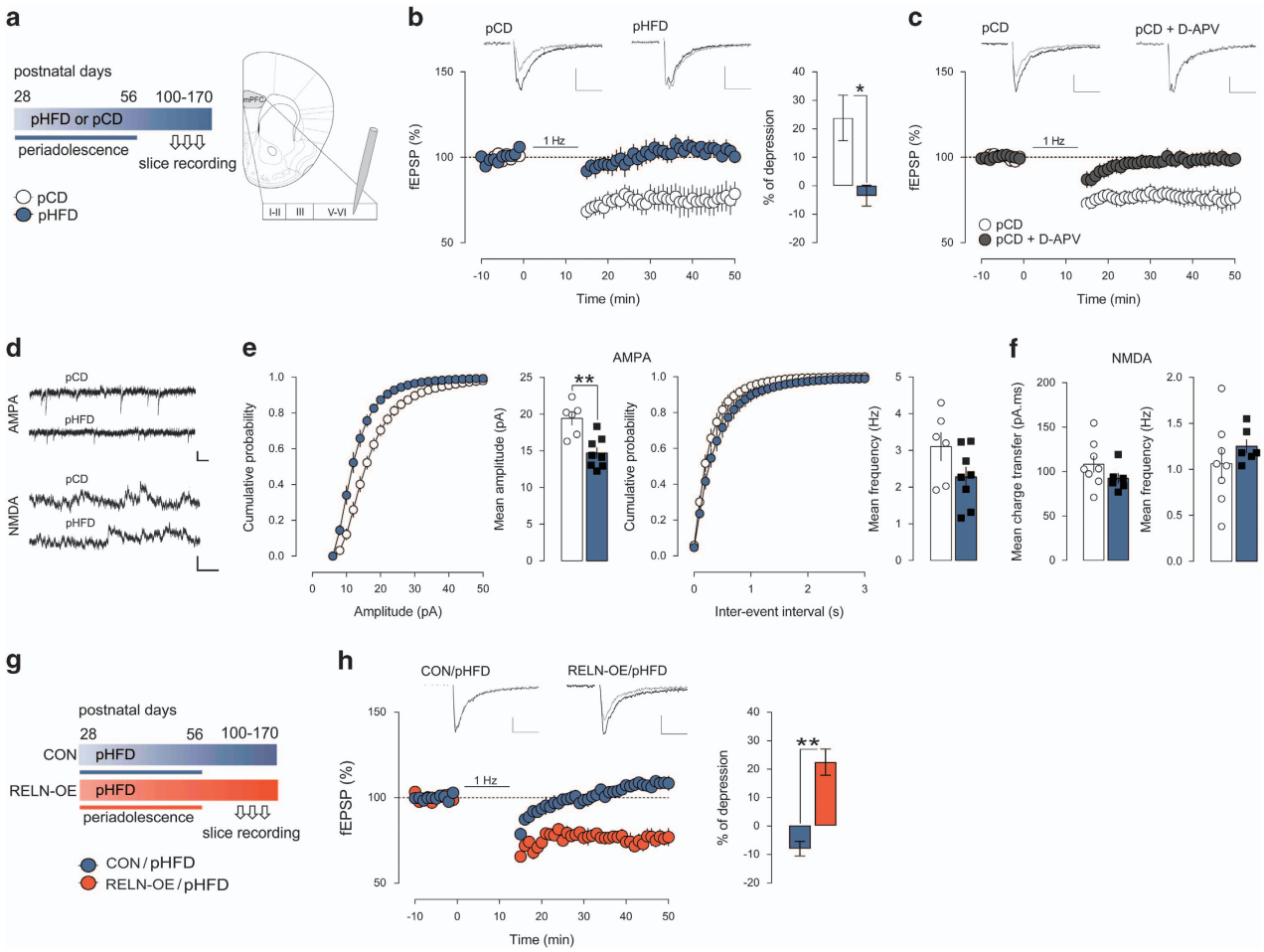


Figure 3. Periadolescent high-fat diet (pHFD) impairs AMPA- and *N*-methyl-D-aspartate (NMDA)-related synaptic functions. (**a**) Experimental design used to assess the effects of pHFD vs control diet (pCD) on physiological properties of medial prefrontal (mPFC) excitatory synapses. See also Supplementary Figure S3 and S4. (**b**) pHFD abolished NMDA-dependent long-term depression (LTD). Left: Grouped time courses of field excitatory postsynaptic potential (fEPSP) responses expressed as the percentage of baseline before and after low-frequency stimulation (LFS) (1 Hz, 15 min). LFS induced LTD of mPFC synapses in pCD mice, whereas no LTD was observed in age-matched pHFD mice. Inset: Representative LTD traces before (in black) and 30 min after LTD induction (in gray). Stimulus artifacts were blanked. Calibration: 0.1 mV, 10 ms. Right: The percentage of depression (pCD: 23.8 ± 8.0% and pHFD: − 3.5 ± 3.6%) was measured at 30–35 min after LFS. (**c**) The 15 min 1 Hz LTD protocol is NMDA-dependent. Grouped time courses of fEPSP responses (percentage of baseline before and after LFS). Inset: Representative LTD traces, as in (**b**). LFS induced LTD of mPFC synapses in pCD mice (24.5 ± 6.3%) and this effect was blocked by the selective NMDA antagonist (2*R*)-amino-5-phosphonopentanoate (D-APV) (1.0 ± 3.8%). (**d**) Representative recordings of AMPA receptor-mediated spontaneous excitatory postsynaptic currents (AMPAR-sEPSCs) (top) and NMDAR-sEPSCs (bottom) taken from pCD and pHFD mice. Calibration AMPA: 10 pA, 100 ms; NMDA: 20 pA, 125 ms. (**e**) Left panel: pHFD reduced the amplitude of AMPAR-sEPSCs as compared with pCD, depicted on a cumulative probability curve (left) and estimated by calculating the mean amplitude of AMPAR-sEPSCs (right) in pHFD (mean amplitude: 14.8 ± 0.8 pA) and in pCD (19.4 ± 0.9 pA) mice. Right panel: pHFD did not affect the frequency of AMPAR-sEPSCs (right) in pHFD and pCD mice (2.3 ± 0.3 vs 3.1 ± 0.4 pA). (**f**) Left: pHFD did not affect the mean charge transfer of NMDAR-sEPSCs as compared with pCD. Right: pHFD did not affect the mean frequency of NMDAR-spEPSCs as compared with pCD. (**g**) Experimental design used to assess the effects of reelin overexpression (RELN-OE) on NMDA-LTD in the mPFC of pHFD mice. See also Supplementary Figure S5. (**h**) RELN-OE prevented the pHFD-induced deficits in NMDA-LTD. Left: Grouped time courses of fEPSP responses (percentage of baseline before and after LFS). As in (**b**), no LTD was observed in control genotype (CON) mice fed HFD (CON/pHFD). In contrast, LFS induced LTD in RELN-OE/pHFD mice. Inset: Representative LTD traces, as in (**b**). Right: The percentage of depression (CON/pHFD: − 8.1 ± 2.6% and RELN-OE/pHFD: 22.3 ± 4.6%) was measured at 30–35 min post-LFS. **P* < 0.05; ***P* < 0.01. All data are means ± s.e.m.

We next ascertained the specificity of the effects of RELN-OE on pHFD-induced behavioral and cognitive abnormalities. We previously showed that pHFD leads to an impairment in sensorimotor gating in the form of a reduced PPI of the acoustic startle reflex, a phenotype that is dependent on accumbal dopamine.[58] Here, we replicate the PPI-disrupting effects of pHFD and further show that this deficit cannot be prevented by RELN-OE (Figure 4h). Similarly, RELN-OE failed to prevent pHFD-induced deficits in hippocampus-regulated[59] expression of contextual fear, a phenotype that is disrupted in diet-induced obesity models[11] (Figure 4i). In the same mice, however, RELN-OE fully prevented deficits in the retention of cued fear extinction (Figure 4i), which critically involves mPFC activity.[59] These results thus suggest that RELN-OE selectively prevents mPFC-related, but not striatal or hippocampal-dependent behavioral abnormalities in mice exposed to pHFD. They also indicate that, although the mPFC-related consequences of pHFD are closely related to loss of RELN, pHFD elicits additional RELN-independent effects within other brain regions.

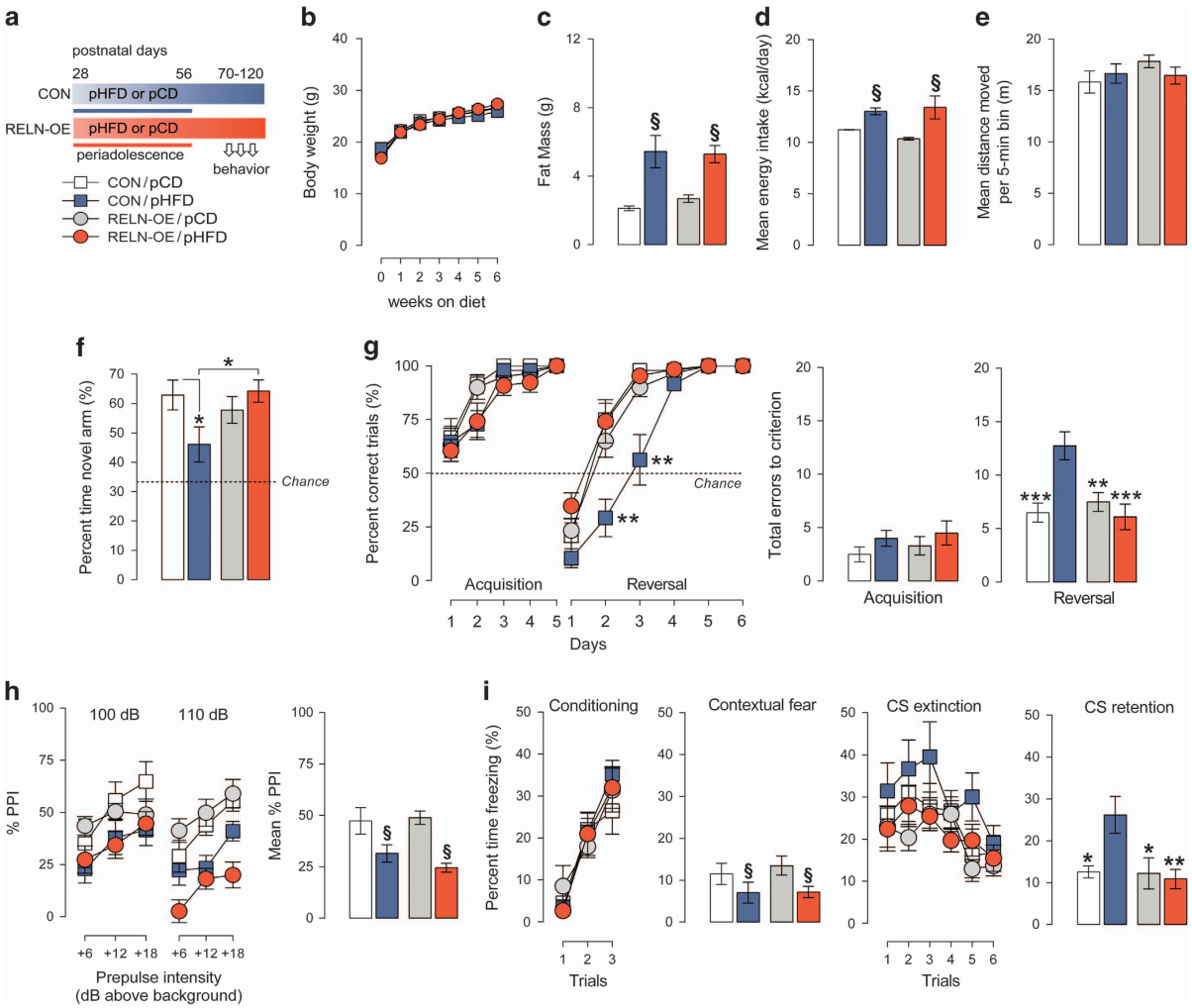


Figure 4. Transgenic reelin (RELN) overexpression prevents prefrontal cognitive dysfunctions induced by periadolescent high-fat diet (pHFD). (**a**) Experimental design used to assess the metabolic and cognitive effects of reelin overexpression (RELN-OE) in pHFD vs control diet (pCD) mice. See also Supplementary Figure S5. (**b**) pHFD did not significantly affect body weights of control genotype (CON) and RELN-OE mice as compared with pCD. (**c**) pHFD significantly increased body adiposity of CON and RELN-OE mice as compared with pCD. (**d**) pHFD increased mean energy intake as compared with pCD in CON and RELN-OE mice. (**e**) pHFD did not affect locomotor activity in a standard open-field arena as compared with pCD (mean distance moved per 5-min bins). (**f**) Working memory as indexed by the percent time spent in the novel arm in a Y-maze spatial recognition paradigm. RELN-OE in pHFD mice ($=64.3\pm3.8\%$ time novel arm) prevented the pHFD-induced working memory deficits displayed by CON/pHFD mice ($=46.1\pm6.0\%$), bringing back performance to CON/pCD levels ($=62.9\pm5.1\%$). (**g**) RELN-OE in pHFD mice ($=6.1\pm1.1$ reversal trials) prevented the pHFD-induced deficits in reversal learning ($=12.8\pm1.3$ reversal trials in CON/pHFD mice), bringing back performance to CON/pCD levels ($=6.5\pm0.9$ reversal trials). Line plot: Percent correct trials during the acquisition and reversal of left-right discrimination in a water T-maze. Bar plot: Total number of errors to criterion during the acquisition and reversal phases. (**h**) RELN-OE did not prevent the pHFD-induced prepulse inhibition (PPI) deficits displayed by CON/pHFD mice compared with CON/pCD mice. Line plot: % PPI as a function of different prepulse intensities (dB above background of 65 dB_A) using 100 and 110 dB_A pulse stimuli. Bar plot: mean %PPI across all pulse and prepulse levels. (**i**) RELN-OE in pHFD mice ($=10.9\pm2.3\%$ time freezing) prevented the pHFD-induced deficits in CS_{tone}-induced retention of extinction behavior in CON/pHFD mice ($=26.2\pm4.4\%$), bringing back performance to CON/pCD levels ($=12.6\pm1.4\%$). Percent time freezing during the initial conditioning phase, contextual fear expression phase, conditioned stimulus (tone) (CS_{tone}) extinction phase and retention of CS_{tone} extinction phase. The dashed lines represent the performance chance level in the respective tests. $^{\S}P<0.05$, 0.01 or 0.001, representing the main effect of diet. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$: *post hoc* effects at individual time points. All data are means ± s.e.m.

Adult intra-mPFC administration of rRELN is sufficient to rescue prefrontal cognitive dysfunctions induced by pHFD

One limitation of the transgenic RELN-OE model is that the expression of the target gene emerges early during postnatal maturation, so that the preventive effects of RELN overexpression could be mediated by subtle RELN-induced alterations in the maturation of prefrontal excitatory synapses[29] rather than by an active and ongoing role of RELN signaling[46] in adult prefrontal functions. To overcome these interpretative limitations, we used a more selective approach and injected a rRELN protein[45] directly into the mPFC several days before cognitive testing according to previous work by Rogers *et al.*[46,60] Consistent with previous findings,[60] we first confirmed that *in vivo*-administered rRELN can rapidly activate phosphorylation of its obligate downstream adaptor protein Dab1 in the mPFC 15 min (Figure 5a) but not 4 days (Supplementary Figure S6) after intraprefrontal injection.[60] In a new cohort of mice (Figure 5b and Supplementary Figure S7), we then verified that rRELN administration *per se* did not affect body weight and basal locomotor activity 3 days after injection as compared with vehicle infusion (Figures 5c and d).

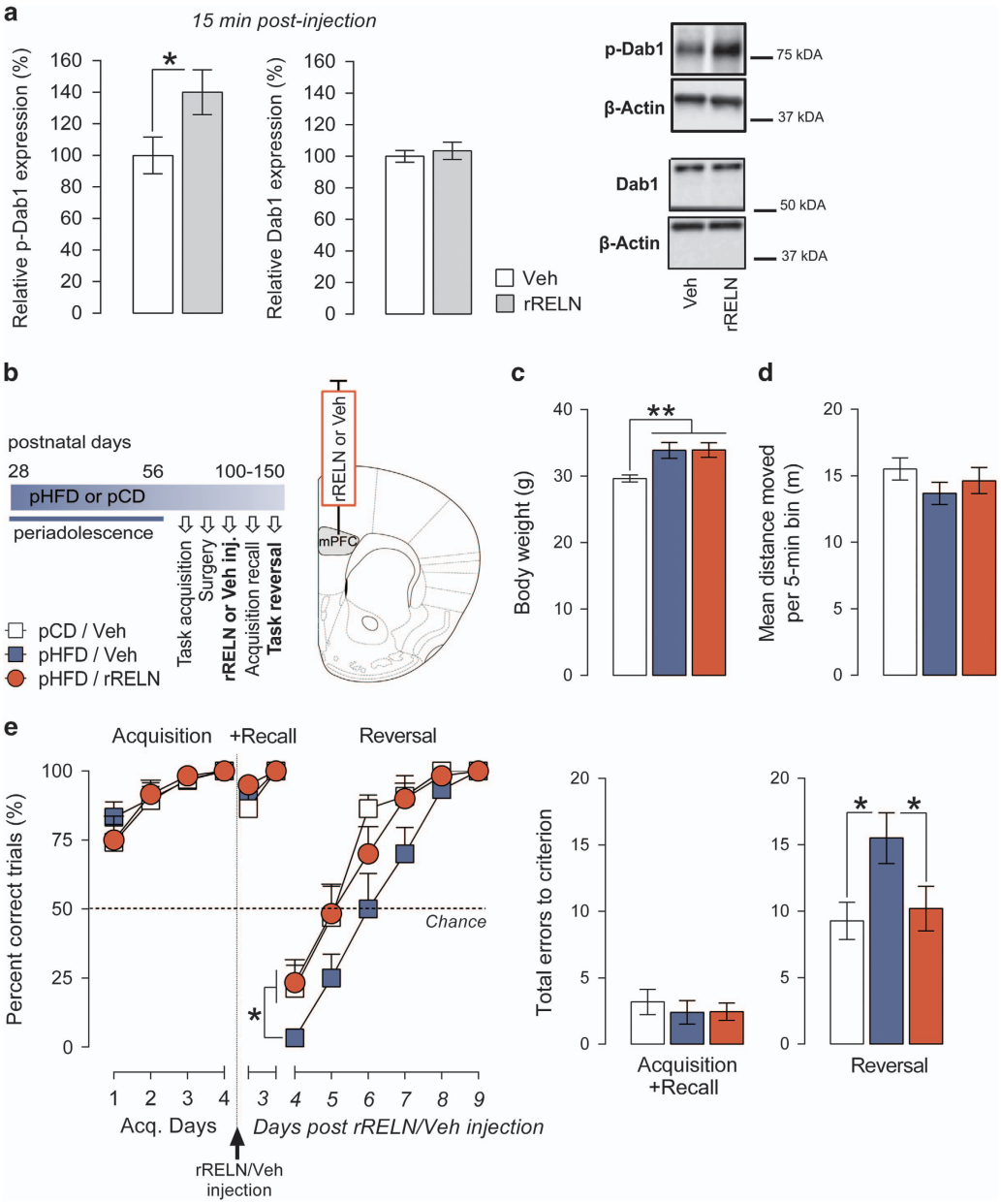


Figure 5. Adult intramedial prefrontal cortex (mPFC) administration of recombinant reelin (rRELN) is sufficient to rescue prefrontal cognitive dysfunctions induced by periadolescent high-fat diet (pHFD). (**a**) Protein expression levels of the obligate downstream adaptor protein disabled-1 (Dab1) and its phosphorylated form p-Dab1 in the mPFC as assessed by western blots in wild-type C57BL/6 N mice 15 min following intra-mPFC administration of rRELN or corresponding vehicle (Veh). The photomicrographs show representative western blot samples for p-Dab1 and Dab1 proteins; β-actin is shown as loading controls for comparisons. See also Supplementary Figure S6. (**b**) Experimental design used to assess the metabolic and cognitive effects of intra-mPFC rRELN or Veh administration in mice exposed to pHFD vs control diet (pCD). See also Supplementary Figure S7. (**c**) pHFD increased body weights to a similar extent in pHFD/Veh and pHFD/rRELN mice as compared with pCD/Veh mice, as assessed on the day of testing (P138). (**d**) rRELN injections or pHFD did not affect the distance moved in a standard Y-maze arena (average of 5-min bins) 3 days after rRELN or Veh injections. (**e**) rRELN injections in pHFD mice ($=10.2\pm1.7$ reversal trials) prevented the reversal learning deficits displayed by pHFD/Veh animals ($=15.5\pm1.9$ reversal trials), bringing back performance to the levels of control pCD/Veh mice ($=9.3\pm1.4$ reversal trials). Line plot: Percent correct trials during the acquisition (done before surgery), recall (day 3 postinjection) and reversal (days 4–9 postinjection) of a left-right discrimination task in a water T-maze. Bar plot: Total number of errors to criterion during the acquisition+recall and reversal phases. The dashed lines represent the performance chance level in the respective tests. *$P < 0.05$: Main effects or *post hoc* effects at individual time points. All data are means $\pm$ s.e.m.

rRELN also did not affect the animals' ability to recall a previously acquired discrimination learning task (Figure 5e, Acquisition recall). Intra-mPFC administration of rRELN, however, fully prevented the pHFD-induced deficits in cognitive flexibility, normalizing performance to control levels (Figure 5e). These data thus further support the hypothesis that RELN deficiency functionally contributes to the emergence of cognitive deficits induced by pHFD. In addition, these findings suggest that the cognitive deficits of pHFD animals are primarily driven by the acute reduction of RELN levels in adulthood—and its established roles in

the modulation of NMDA-dependent synaptic plasticity[46,54]—rather than being mediated by subtle developmental effects of impaired RELN function across periadolescent mPFC maturation.[29,31]

DISCUSSION

The past few decades have witnessed a marked shift in the quality of human nutrition with the emergence of nutritionally poor fat-rich diets, in particular within the Western hemisphere.[1–3] This issue is of upmost importance for public health given the widespread nature of this phenomenon.[3] Although the deleterious effects of HFD on metabolism have been well characterized,[5] their potential negative impact on mental health remains ill-defined. Indeed, although previous studies have shown that HFD can undermine hippocampal and prefrontal cognitive functions,[6,9–15] the neuronal mechanisms and windows of vulnerability remained largely unknown thus far.

The periadolescent period is known to represent an environmentally sensitive time window for the mPFC.[17,19,20,61,62] Whilst this notion primarily stems from work on drug exposure or psychosocial stress,[17,19,20,61,62] less attention has been paid to the potential negative effects of other daily-life environmental factors such as poor nutrition. Although previous human studies have provided circumstantial evidence for a role of nutrition in adolescent mPFC functions,[21,22,25] we believe the present mouse study provides the first direct evidence demonstrating hypervulnerability of the adolescent mPFC towards nutritional stress in the form of HFD exposure. Our findings highlight that excessive HFD intake during adolescence is an environmental disruptor of the adult mPFC and suggest that a careful nutritional balance during this sensitive period is pivotal for reaching the full capacity of adult prefrontal functions.[63]

The present study also provides several complementary lines of evidence indicating that adolescent neuronal hypervulnerability towards HFD is mediated by a loss of the RELN protein. Indeed, we show that RELN-expressing interneurons in the mPFC represent selective targets of HFD exposure. This effect is highly specific to the periadolescent period and likely emerges as a result of the immature character of the mPFC during this time window.[19] We believe that these results are of significance because they extend ongoing work on the neurobiology of sensitive periods in the mPFC. Previous studies have indicated that PV fast-spiking interneurons critically contribute to the emergence of prefrontal cortical impairments following adolescent exposure to certain environmental stressors such as oxidative stress or drugs of abuse.[62,64] Our present findings suggest that RELN neurons might provide yet another route of vulnerability that drives prefrontal pathology in early-environmental stress models.[36,37] This notion aligns with previous work showing that corticosterone treatment during adolescence significantly affects RELN/NMDA prefrontal cortical systems.[38] Thus, various interneuron populations might individually contribute to neurodevelopmental hypervulnerability depending on the nature of the environmental stimulus and age of exposure.

In view of the potent and selective effects of pHFD on RELN neurons, we also identified a synaptic signature of mPFC RELN deficiency. We show that the pHFD-associated RELN impairment, as predicted from previous work,[34] is associated with blunted NMDA-LTD functions. The latter are likely driven by abnormal AMPA neurotransmission (as illustrated by reduced AMPAR-sEPSC amplitude) and are expected to contribute to the cognitive anomalies of pHFD. Indeed, deficits in NMDA-LTD on the one hand, and in AMPA internalization on the other hand, were previously shown to promote cognitive flexibility or working memory impairments in several genetic models.[55–57] Interestingly, we also show that NMDAR-sEPSCs remain unaffected by adolescent HFD exposure. Hence, this nutritional manipulation spared at least some of the synaptic functions typically regulated by RELN.[28,29,31–35,54] Although we do not have a parsimonious explanation as to why AMPAR- but not NMDAR-sEPSCs are affected, it is possible that distinct modalities of RELN action are impaired depending on the precise environmental or genetic conditions.[33] This could possibly arise from a differential modulation of Src-kinase vs phosphoinositide 3-kinase signaling pathways, which have previously been shown to mediate changes in NMDAR-sEPSCs vs AMPAR-sEPSCs, respectively, following RELN action.[35]

Our study further demonstrates that RELN deficiency induced by pHFD does not only represent an epiphenomenon, but, in fact, critically drives the appearance of prefrontal pathology following adolescent HFD exposure. We first show that transgenic overexpression of RELN prevents the emergence of electrophysiological deficits (NMDA-LTD) in pHFD animals. Although we acknowledge that additional control groups would have been helpful for the LTD experiment, we later extend these experiments using a full-factorial experimental design. We show that RELN-OE fully prevents prefrontal-dependent (but not prefrontal-independent) cognitive deficits while at the same time RELN-OE has no effect in control animals. Intriguingly, we also reveal that adult intra-mPFC administration of rRELN is sufficient to restore mPFC-regulated cognitive functions. These latter findings thus suggest that the cognitive impairments manifest in pHFD animals are primarily a result of impaired RELN-mediated signaling in the adult brain, rather than a consequence of deficient RELN functions during periadolescent mPFC maturation.[29,31] Based on elegant work from Rogers *et al*.,[46,60] it appears plausible that the potentiating effects of rRELN on cognition are likely mediated via its long-term consequences (i.e. several days) on synaptic plasticity, possibly through its effects on molecular pathways that govern NMDA-LTD at excitatory synapses. Hence, the procognitive effects of rRELN are independent of any ongoing (short-lived) effects on Dab1 phosphorylation (see Supplementary Figure S6). Besides providing mechanistic insights, our data also emphasize a potential therapeutic use of purified RELN to rescue cognitive impairments in the adult subject, in line with previous work in heterozygous RELN-deficient mice.[46] Future work should also attempt identifying alternative ways to protect or promote RELN function in the adult brain, for example, by targeting the mechanisms that regulate RELN expression or degradation.

Although genetic or recombinant augmentation of RELN prevented the cognitive deficits in pHFD mice, it did not affect basic prefrontal cognitive functions *per se*. These results are in line with the notion that RELN haploinsufficiency alone may not be enough to produce overt cognitive impairments, at least in adult animals,[30,50,51] and that additional factors, such as β-amyloid neurotoxicity[40] or periadolescent nutritional stress, are required. Such findings further emphasize that RELN might represent a marker of cognitive or behavioral resilience,[40–42] the loss of which prevents the ability of the brain to cope with cellular or external stressors.

Against these backgrounds, we deem our findings relevant for obese patients, in which excessive consumption of unhealthy dietary fats are a common feature.[65] Indeed, obese patients are at risk of developing neurological comorbidities such as mild cognitive impairment,[66] but the pathophysiological mechanisms remain elusive.[9,67,68] Our study contributes to the understanding of such epidemiological associations and provides mechanistic insights into how prefrontal cognitive dysfunctions might emerge in obese individuals consuming excessive HFD. Interestingly, in our pHFD model, prefrontal RELN deficits appeared after only 4 weeks of HFD exposure, before any changes in body weight. Adult-exposed HFD animals also gained significantly more body weight than periadolescent-exposed animals, yet did not develop prefrontal cognitive deficits following a 6-week dietary exposure. Taken together, these data suggest that HFD consumption, rather

than obesity itself, drives the emergence of prefrontal pathology in periadolescent subjects. Our work also extends previous findings showing that immature regions of the brain display high vulnerability towards the neuronal effects of early-postnatal HFD exposures.[10,58] Therefore, it likely has relevance for public health given the emergence of unhealthy dietary patterns and the alarming prevalence of adolescent obesity in the western hemisphere.[1,2,69,70]

The identified link between pHFD and RELN deficits may also have relevance for psychiatric disorders that involve abnormal RELN expression and associated synaptic defects. One example is schizophrenia, a chronic psychotic disorder that is associated with metabolic and nutritional comorbidities.[71] Excessive HFD intake might exacerbate the RELN and synaptic deficits in patients with schizophrenia[72,73] and/or aggravate cognitive anomalies. This hypothesis was previously formulated by others[7,71] and is based on work showing that schizophrenic patients with metabolic syndrome perform significantly worse than patients without metabolic syndrome on tests of executive function.[74] In addition, our findings may be relevant for the RELN-related neuropathology of AD[75] for which unhealthy dietary fats are an established risk factor.[8]

In conclusion, our findings (summarized in Supplementary Figure S8) highlight that adolescence is associated with an increased vulnerability for mPFC-regulated cognitive deficits induced by nutritional stress in the form of HFD and indicate that the RELN protein is a critical determinant of this pathological association. Our findings are potentially relevant for a broad number of metabolic and neuropsychiatric disorders and argue for a careful consideration of nutritional balances across periadolescent maturation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

MAL designed the study, analyzed data, wrote the original manuscript and performed the behavioral, surgical, metabolic and imaging experiments. OL performed and analyzed the electrophysiology experiments. JR and MAL did the western blots. UW contributed to behavior and surgeries. JI developed the LTD protocol. TN, TG and MAL did the immunostainings. LP and ES generated the transgenic mice and contributed to writing. AR, CL and WL contributed to data interpretation and writing. PC and UM designed and supervised the entire study, analyzed data and wrote the manuscript.

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)