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Long-Term, Fructose-Induced Metabolic Syndrome-Like Condition Is Associated with Higher Metabolism, Reduced Synaptic Plasticity and Cognitive Impairment in *Octodon degus*

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Abstract

There has been a progressive increase in the incidence of fructose-induced metabolic disorders, such as metabolic syndrome (MetS). Moreover, novel evidence reported negative effects of high-fructose diets in brain function. This study was designed to evaluate for the first time the effects of long-term fructose consumption (LT-FC) on the normal ageing process in a long-lived animal model rodent, *Octodon degus* or degu. Moreover, we could replicate human sugar consumption behaviour over time, leading us to understand then the possible mechanisms by which this MetS-like condition could affect cognitive abilities. Our results support that 28 months (from pup to adulthood) of a 15% solution of fructose induced clinical conditions similar to MetS which includes an insulin-resistance scenario together with elevated basal metabolic rate and non-alcoholic fatty liver disease. Additionally, we extended our analysis to evaluate the impact of this MetS-like condition on the functional and cognitive brain processes. Behavioural test suggests that fructose-induced MetS-like condition impair hippocampal-dependent and independent memory performance. Moreover, we also reported several neuropathological events as impaired hippocampal redox balance, together with synaptic protein loss. These changes might be responsible for the alterations in synaptic plasticity and transmitter release observed in these cognitively impaired animals. Our results indicate that LT-FC induced several facets of MetS that eventually could trigger brain disorders, in particular, synaptic dysfunction and reduced cognition.

Keywords *Octodon degus* · Fructose · Insulin resistance · Basal metabolic rate · Non-alcoholic fatty liver · Behaviour performance · Synaptic plasticity

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Introduction

Fructose is a monosaccharide found in many food sources, such as honey, fruits, and some root vegetables, furthermore constitutes a main component of the human diet [1, 2]. The largest source of fructose consumption is in the form of sweetening agent of soft drinks particularly in carbonated beverages, which constitutes the most popular refreshments among much of the world's population [3]. Whether sweetened with sucrose (50% fructose) or high fructose corn syrup, HFCS (either 42 or 55% fructose), the fructose content of beverages sweetened with sugars ranges from 7 to 15% by weight [3, 4]. The resulting per capita increase of fructose in the past three decades has been proposed as a major contributor to the increased prevalence of obesity and consequent metabolic disorders [3–5].

Fructose is preferentially metabolized in the liver favouring hepatic de novo lipogenesis, which contributes to weight gain, dyslipidemia, insulin resistance [6, 7], and, as found lately, non-alcoholic fatty liver disease (NAFLD) [8]. The coexistence of some of these abnormalities is often categorized as metabolic syndrome (MetS) [8]. MetS refers to a cluster of cardiometabolic risk factors (obesity, insulin resistance, hypertension, and dyslipidemia) unequivocally linked to an increased risk of developing atherosclerotic cardiovascular disease (ASCVD) and type 2 diabetes mellitus (T2DM). Although the concept of a "syndrome" has been debated, MetS is clinically useful for identifying people who are at risk of these complications, although it remains unclear whether the syndrome as a whole confers more risk than the sum of its parts [9].

Furthermore, some clinical studies have associated MetS with an increased risk of neurological disorders, such as Alzheimer's disease [9, 10]. In addition, there is evidence to indicate that the effects of high fructose consumption directly affect the brain function [11, 12], causing negative alterations in hippocampal-dependent memory [13–15] and accelerates the normal aging process [16]. MetS currently has become a worldwide disease affecting patients of all ages, sexes, and races and resulting in high health care costs [14, 17].

The concern of dietary fructose and its possible effects on metabolic disorders and health is argument by the experimental conditions (i.e. effects of dose, duration of treatment, the mode of fructose delivery and the animal model) [5, 18, 19]. Many studies of the adverse effects of fructose tend to exaggerate fructose dosing protocols, exceeding the population intake found in many human studies by at least 3-fold; doses are sometimes even more extreme in animal models, reaching levels of 4- to 5-fold excess and therefore exceeding total carbohydrates consumption [20]. If these extreme doses produce biochemically significant shifts in short periods of time, these studies have low physiological value and their outcomes cannot be relied upon for assessing human risk or making public health recommendations [19, 21].

Several studies reported conflicting results, in part explained by variations in what the researchers define as prolonged consumption of fructose or the mode of incorporating fructose into the meal [5, 19]. If well fructose consumption would lead to a positive energy homeostasis independent of mode of ingestion, some authors observed opposite results when administrated fructose in aqueous form (i.e. significant long lasting effects of fructose consumption as drinks or liquid meals), or in food (i.e. no long lasting effects of fructose consumption as solid foods, with or without fructose in drink) to better control and monitor caloric intake [8, 22, 23]. Moreover, if well, studies of longer duration are better understand the implications of the effect of fructose consumption on different aspects of metabolism in terms of possible risk to health, they are logistically challenging due to high cost and waning compliance with longer duration of follow-up [4, 7].

Another major limitation to the study of the pathogenesis of metabolic disorders in humans is the lack of relevant animal models with which to carefully investigate the biological mechanisms underlying the effects of sugar consumption on energy balance and its relationship to metabolic disease [24]. In previous years, the native rodent species of central Chile, the degu (*Octodon degus*), has been proposed as a well suited model to study diet-induced disorders [25, 26]. Degus has a predisposition to develop non-obese diabetes that is often accompanied by cataracts when regularly fed a sugar-containing diet [9, 27, 28]. Molecular and histological aspects indicate that the strong predisposition of degus to diabetes is due to their low metabolic activity and reduced insulin receptor binding affinity, thus causing a natural resistance to insulin [29, 30]. Additionally, its lifespan is considerably longer than those of the other experimental animal models [31]. Then, degus has great potential as a suitable model for long-term dietary and aged-related studies and compared to those described in the humans [31, 32].

In this study, we tested for the first time the effects of long-term fructose consumption (28 months) on the normal ageing process in the long-living animal model, *O. degus*. The consequences of longer period of fructose consumption were assessed by measuring a set of clinical criteria associated to MetS (i.e. insulin resistance, basal metabolic rate, and non-alcoholic fatty liver disease). Additionally, we extended our analysis to evaluate the impact of MetS-like condition on the brain through estimations of several behavioural, electrophysiological, and biochemical variables. We hypothesized that degus consuming fructose concentration at 15% for a prolonged time (from pup to adulthood, 28 months) would develop a MetS-like condition, including impaired hippocampal-dependent memory in comparison to control degus (0% fructose). To the best of our knowledge, this study is the first to evaluate the effects of fructose administered over a prolonged period in this long-lived rodent model. We believe that degus is a good model of maintained but not overburdened fructose consumption replicates human sugar

intake behaviour over time, which is important because MetS appears after a long period of poor dietary habits and not after binge eating.

Methods

Animals and Experimental Procedure

Male degus of 4 months old were obtained from our colony. These animals were all derived from laboratory-bred lines. The degus were randomly divided into two groups ($n = 10$ and $n = 12$ per group) and kept in pairs of related and unrelated males housed in clear acrylic aquaria (length × height × depth: 50 × 35 × 23 cm) with a bedding of hardwood chips. Each cage contained one nestbox made of clear acrylic (22 × 12 × 15 cm). The animals were kept in a ventilated room and exposed to a natural photoperiod (12 h light/dark cycle) and ambient temperature (yearly minimum = 13.4 ± 0.2 °C; yearly maximum = 24.9 ± 0.2 °C). All studies followed the guidelines of the National Institutes of Health (NIH, Baltimore, MD, USA). All procedures were approved by the Bioethical and Biosafety Committee of the Faculty of Biological Sciences of the Pontificia Universidad Católica de Chile (CBB-121-2013). All efforts were made to minimize animal suffering and to reduce the number of animals used.

All animals were fed a standard rabbit commercial pellet diet (Champion, Santiago, Chile). The chemical composition of the diet was dry matter = 90.6%, ash = 10.8%, crude fibre = 16.5%, neutral detergent fibre = 37.8%, acid detergent fibre = 19.8%, lipids = 3.0%, proteins (N × 6.25) = 20.0%, carbohydrates = 40.3%, and total energy content = 18.4 ± 0.5 kJ g^{-1} [33]. The control group ($n = 12$) was provided tap water whereas the experimental group ($n = 10$) was provided a fructose solution in water at a concentration of 150 g L^{-1} (15% solution). This fructose dose tends to mimic the highest HFCS concentration of U.S. soft drinks [3]. For 28 months, the degus were allowed free access to the pellets and water or fructose solution. At the end of the experiment, male degus weighted 220 ± 4 g (mean ± standard error of the mean).

Body Weight, Blood Glucose Levels and Blood Pressure

Each month, we registered body weight gain and blood glucose levels with a glucometer (OneTouch Ultra2; LifeScan Inc., Milpitas, CA, USA) in degus fasted overnight. Fructose and water intake were recorded weekly by measuring the remnants of a total volume of 500 mL. The caloric intake was calculated based on the fluid intakes (4 kcal/g). Systolic blood pressure was measured only once at 19 months after beginning the treatment (Fig. 1a) in conscious degus using tail-cuff plethysmography with a Grass polygraph. Fasting degus (12–15 h) were euthanized by decapitation at the end of the behavioural tests (described later) after isofluorane deep anaesthesia. Trunk blood was collected immediately and centrifuged at 3500×*g* for 10 min to collect plasma, which was stored at −150 °C until the determination of chemistry parameters. Additionally, the liver was immediately removed and weighed. A portion of liver tissue was fixed in 10% formalin for histopathological analysis (see below).

Blood Chemistry

At the end of experiment, plasma biochemistry parameters were recorded (Fig. 1a). Plasma glucose and triglycerides were measured using the glucose oxidase and peroxidase methods with quinonimine as a chromogen. The amounts of plasma glucose and triglycerides are related to the amount of quinonimine, which was measured by spectrophotometry at 500 nm [34]. Fasting plasma insulin concentrations were measured by a solid-phase, two site chemiluminescent immunometric assay (Immulite/Immulite 1000; Siemens). Plasma fructosamine levels were assayed by the method described by Johnson et al. [35]. In brief, the assay was based on the ability of ketoamine to reduce nitroblue tetrazolium (NBT; Sigma Chemical Co., St. Louis, MO) to a tetrazindyl radical NBT measurable at 530 nm. The homeostatic model assessment (HOMA) of insulin resistance was calculated as follows: $HOMA_{IR}$ = fasting glucose (mg dL^{-1}) * fasting insulin (μU mL^{-1})/405 [36].

Blood Glucose Tolerance Test (GTT)

To evaluate the capacity of degus to regulate their blood glucose levels, we performed GTTs. At 27 months after beginning the treatment (Fig. 1a), fasting animals received an intraperitoneal injection containing 40% glucose (2 g kg^{-1} of body weight). Blood samples of approximately 2 μL were collected from the saphenous vein after 0, 30, 60, 120 and 180 min for blood glucose concentration measurements using a glucometer (OneTouch Ultra2; LifeScan Inc., Milpitas, CA, USA).

To evaluate the normality of the blood glucose levels, we followed the National Diabetes Data Group criteria, [37]; i.e. 2 h after loading, blood glucose must be lower than 7.78 mmol L^{-1}, and after 30, 60, and 90 min, less than 11.11 mmol L^{-1} is considered normal. These criteria constitute de most exigent standard reference for glucose concentration values reported for non-human mammals, including rodent species [29].

Basal Metabolic Rate Records

As degus are diurnal, basal metabolic rate (BMR) was determined using a standard flow-through respirometry system during the inactive phase (1900 h–0800 h), in a post-absorptive state (animals were fasted for 8 h) and within the thermoneutral

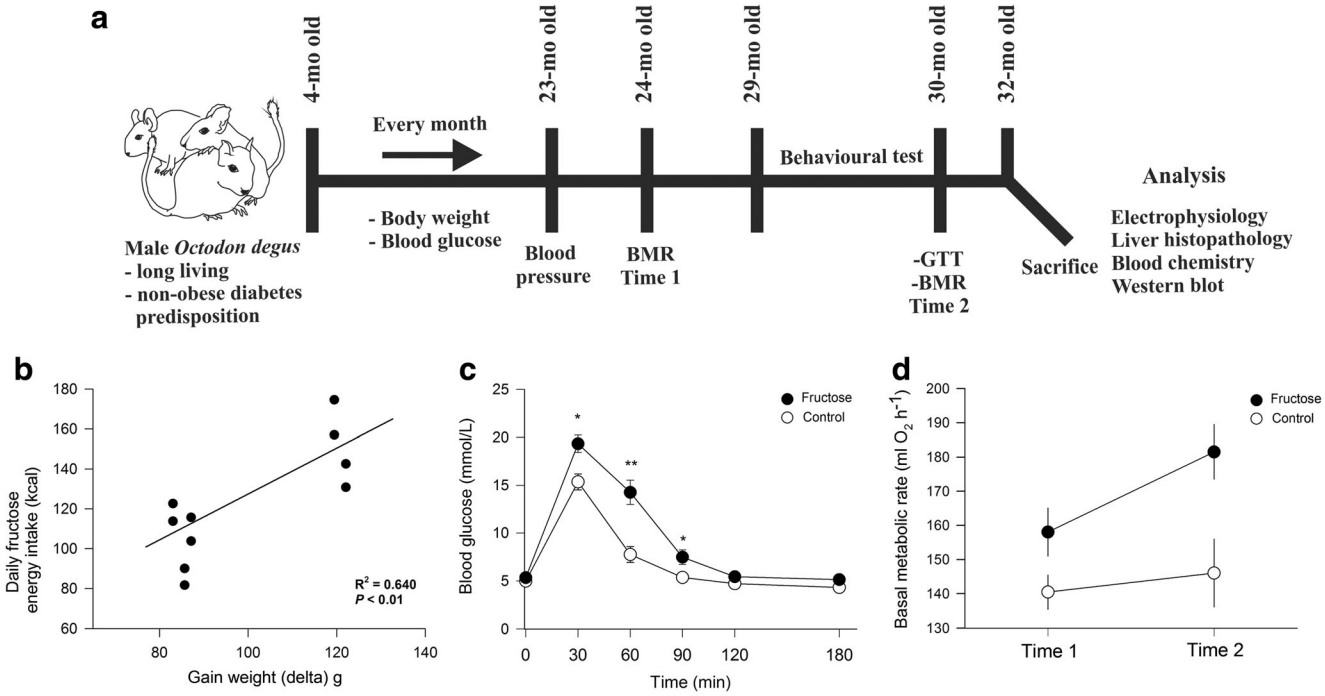

Fig. 1 Scheme of experimental design. **a** A fructose solution in water at a concentration of 150 g L^{-1} (15% solution) was administrated at 28 months (from 4 months old until 32 months old). Monthly, we controlled by body weight gain and blood glucose level. At 19 months after beginning the treatment, the systolic blood pressure was recorded. One month later, BMR time 1 was measured. Behavioural test was recorded between 25 and 26 months after beginning the treatment. One month later, GTT and BMR time 2 were measured. Later, we wait 2 months after the end of behaviour test to sacrifice all animals for electrophysiological, histological and biochemical analyses. Effect of chronic fructose energy intake on **b** gain weight over the course of 28 months explained by the daily caloric intake in the fructose-fed degus. **c** GTT results of the control and fructose-fed groups ($n = 9$ and 6 animals per group, respectively. Animals were fasted overnight (15 h), d-glucose (2 g kg^{-1}) was administered by intraperitoneal injection, and blood glucose levels were determined at the indicated times using a glucometer (**d**) comparison of the BMR among the control and fructose-fed groups over the course of 28 months. For BMR time 1, 10 animals were used per group. Whereas for BMR time 2, we used 11 and 8 animals for control and fructose-fed group, respectively. Values are shown as the mean ± SEM. The data were analysed statistically using one-way ANOVA followed by Tukey's post hoc test. Asterisk indicates significant differences between both groups. $*p < 0.05$; $**p < 0.01$

zone of this species [33, 38]. Rodents were weighed using an electronic balance (± 0.1 g), placed in a metabolic chamber (1000 mL) and then placed in a controlled-temperature cabinet (Sable Systems, Henderson, NV, USA) at 30 ± 0.5 °C [39]. The metabolic chamber received air that passed through CO_2 water absorbent granules (Baralyme and Drierite, respectively) at 800 mL min^{-1} from a mass flow controller, which was sufficient to ensure adequate mixing in the chamber. The excurrent air passed again through Baralyme and Drierite columns before passing through a Foxbox model O_2 analyser (Sable System, Nevada, USA), which was calibrated with a known mix of oxygen (20%) and nitrogen (80%) certified by chromatography (INDURA, Chile). Air was sampled every 5 s by the O_2 analyser. The mass flow meter was calibrated with a volumetric (bubble) flow metre. Rodents remained in the metabolic chamber for at least 12 h until a visual inspection of the recorded data allowed for the determination that steady-state conditions had been reached. Because water vapour and CO_2 were scrubbed before entering the O_2 analyser, oxygen consumption was calculated as shown by Withers, [40]: $VO_2 = [FR*60*(Fi\ O_2 - Fe\ O_2)]/(1 - Fi\ O_2)$, where FR is the flow rate in mL min^{-1}, and Fi and Fe are the fractional concentrations of O_2 entering and leaving the metabolic chamber, respectively. Output from the oxygen analyser (%) and the flow metre was digitalized using a Universal Interface II (Sable Systems, Nevada, USA) and recorded on a personal computer using EXPEDATA data acquisition software (Sable Systems, Nevada, USA). Following standard protocols [38], we averaged the minimum VO_2 records over a 10-min period. The BMR records were registered twice over the 28 months of fructose intake (Fig. 1a): namely, time 1 (20 months after beginning the treatment) and time 2 (at the end of the treatment).

Liver Histopathology

Liver sections from the two groups were routinely fixed in 10% formalin and embedded in paraffin. Haematoxylin and eosin staining was then performed per the standard procedures. Sirius red was used to assess the grade of fibrosis (collagen deposition). An expert pathologist blinded to the assigned experimental groups assessed liver histology and scored the samples per the degree of steatosis and

inflammation according to the NAFLD activity score (NAS) [41, 42]. Briefly, the NAS was assigned as follows: (i) steatosis: grade 0, < 5%; grade 1, steatosis of 5–33% of the parenchyma; grade 2, steatosis of > 33–66% of the parenchyma; grade 3, steatosis of > 66% of the parenchyma, (ii) inflammation: grade 0, no inflammatory foci per high-power field (HPF); grade 1, 1–5 inflammatory foci per HPF; grade 2, > 5 inflammatory foci per HPF, (iii) ballooning: grade 0,no ballooning; grade 1, few ballooning cells; and grade 3, many cells/prominent ballooning [41, 42].

Behavioural Test

The behavioural performance of degus was determined at 26 months after beginning the treatment (Fig. 1a). Animals were subjected to four behavioural trials, as detailed below. To minimize the effects of behavioural experiences on the results, the experiments were conducted in the order from the less to the more intrusive. The order of experiments was as follows: (i) the open field test; (ii) the social interaction test; (iii) the novel object recognition (NOR) test; and (iv) the Barnes maze test. The animals were subjected to one test per day (except the Barnes maze test, which is longer). All behavioural tests were performed during the day time (between 0900 and 1600 h).

Open Field Test

Animals were observed for 5 min during the open field test. The open field arena consisted of a white Plexiglas box (length × height × depth: 100 × 100 × 100 cm). The number of central crossings (with a four-paw criterion) was scored. In addition, the speed and total distance travelled was assessed [43]. At the end of each session, the animals were returned to their home cages, and the area was wiped clean with a 70% ethanol solution.

Social Interaction Test

The open field arena was subdivided into three equal compartments by means of transparent Plexiglas walls, each with a small opening (diameter, 2.8 cm), allowing access into each compartment. The degus to be used as social partners were sex-matched, unfamiliar, and unrelated. The test comprised three 20-min sessions. The social test was performed following the protocol described by Rivera et al. [43]. Briefly, the three sessions were performed in the following order: Habituation: the test animal was placed in the middle compartment and allowed to explore the apparatus. Session 1 ("Partner I"): the subject was returned to the middle compartment. Simultaneously, the first social partner was placed inside a wire containment cup located in one of the side chambers. The test animal was then free to interact with the social partner or with the empty cup. At the end of session 1, the test animal was returned to his home cage for 1 h, and the area was wiped clean with a 70% ethanol solution to remove odours. Session 2 ("Partner II"): The test animal was again returned to the middle compartment, and a second unfamiliar, unrelated degu was placed inside an identical wire containment cup in the opposite side chamber, which had been empty during session 1. In this part, the test animal had a free choice between the first, already-investigated, unfamiliar degu (partner I) and a novel unfamiliar degu (partner II). As measurements of social interaction, we recorded the frequency of the following behaviours: investigation of the partner (i.e. the number of direct contacts (touches to the cup box with the forepaw or nose) between the test subject and the containment cup housing or not housing the new partner for each chamber individually. To evaluate differences in social recognition, the recognition index (RI) for session 1 was defined as the quotient of the time the degu spent with partner I divided by the sum of the time spent with partner I and the empty cup. For session 2, the RI was calculated as the time spent with partner II divided by the sum of the time spent with partners I and II. A recognition ratio of 0.50 indicates that there was an absence of social motivation and affiliation for session 1 and an absence of social novelty recognition for session 2.

NOR Test

This test arena used an open box (length × height × depth: 63 × 40 × 30 cm) made of white Plexiglas. For this test, we followed the object recognition protocol used for degus by Rivera et al. [43]. Briefly, animals were exposed to a 10-min familiarization assay and then subjected to two consecutive 5 min assays separated by a 1 h inter-trial interval. Session 1 (Familiarization): two objects ("Object A" and "Object B") were placed in the corners of the home cage, and the animal was allowed to freely explore the field for 10 min. Following this period, the objects were removed from the cage and wiped with a 70% ethanol solution, and the test animal was returned to his home cage for 1 h. Session 2 (novel location recognition, NLR): one of the familiar objects (Object B) was moved to an adjacent unoccupied corner. The test animal was then free to interact with the objects for 5 min. Following this period, the objects were removed from the cage and wiped with a 70% ethanol solution, and the test animal was returned to his home cage for 1 h. Session 3 (NOR): one of the familiar objects (Object B) was replaced by a different object. We recorded the familiarization and testing times, and the time spent exploring each object. "Exploration" time was defined as time spent approaching to within 1–3 cm of the object. To quantify NLR and NOR, a RI was calculated as the time spent with Object B divided by the sum of the time spent with Objects B and A.

Barnes Maze Test

The Barnes maze consisted of a circular, 160 cm diameter, elevated platform made of white Plexiglas surrounded by a 45-cm high wall. Eighteen circular holes (8 cm in diameter) were bored

through the platform equidistant from each other (16 cm) and 5.5 cm from the outer edge. All holes except the target hole were blocked. A plastic escape box (length × height × depth: 31 × 13 × 16 cm) was positioned under the escape hole. Accurate performance requires subjects to learn and remember the location of the escape hole; therefore, spatial cues (combinations of different colours and shapes: a yellow star, a red square, and a green apple) were placed on the wall of the maze [44]. This test has strong spatial and hippocampus dependent components [45]. Briefly, the procedure was divided into 3 phases: the habituation, training, and testing phases, following the methods described by Rivera et al. [43]. Session 1 (habituation) began with placing the animal in the escape box for 2 min. The animal was then placed near the escape hole and left for 1 min to escape. If the animal did not enter the escape box, it was gently picked up and helped through the target hole into the escape box, where it was left for 2 min. Finally, the animal was placed in the centre of the maze and left for fourmin to explore the platform and enter the escape box. If the animal did not enter the escape box, it was placed into the escape box as described above and left there for 2 min. In session 2 (training), 2 days after session 1, we trained each animal for 7 days. In session 3 (testing), 7 days after session 2, we exposed the test animals to a memory-retrieval session. Both the training and the testing phases consisted of four consecutive 4 min trials separated by a 5 min resting phase in the animal's homecage. At the beginning of each trial, the animal was confined for 30 s in a start box in the centre of the maze. If the animal did not enter the escape box within the allotted time, it was manually picked up and placed in the escape box, where it remained undisturbed for 2 min. The surfaces of the maze platform were cleaned with 70% ethanol between trials. We recorded the latency to the first visit of the escape hole and the percentage of time spent in the quadrant of the escape hole. We also analysed reference memory errors (each first visit to a non-escape hole in each trial) and working memory errors (repeated visits to the same non-escape hole in the same trial). Additionally, we calculated the reference memory errors per omission error and the working memory errors per omission error, whereby the number of visits to the escape hole without escape was scored as an 'omission error'. To discard locomotors differences between groups, we measured the speed and the distance (in metres) covered from the initiation of escape hole exploration to escape hole entrance.

In all cases, a digital video camera (LifeCam Studio Full HD, Microsoft Corp., Redmond, WA) was mounted above the test arena, and the performance of each animal was monitored using image tracking software (HVS Image, Hampton, UK).

Electrophysiological Records

After 2 months of the end of behavioural test (Fig. 1a), the hippocampus of control and fructose-fed degus ($n = 3$ respectively) was promptly removed and sectioned into 350-μm-thick slices using a vibratome (Leica VT1000S) in ice-cold dissection buffer (5 mM KCl, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 212.7 mM sucrose, 10 mM dextrose, 3 mM $MgCl_2$, and 1 mM $CaCl_2$, equilibrated with 95% O_2 and 5% CO_2). The slices were transferred and maintained for 1 h at room temperature in normal artificial cerebrospinal fluid (ACSF), which was similar to the dissection buffer, but with the sucrose replaced by 124 mM NaCl, the $MgCl_2$ lowered to 1 mM, and the $CaCl_2$ increased to 2 mM. All recordings were performed in a submersion-recording chamber perfused with ACSF (room temperature; 2 mL min^{-1}). Field excitatory postsynaptic potentials (fEPSPs) were evoked by stimulating the Schaffer collaterals with 0.2 ms pulses delivered through concentric bipolar stimulating electrodes (FHC) and recorded extracellularly in the CA1 stratum radiatum. Basal synaptic transmission was assayed by determining the input/output (I/O) relationships of the fEPSPs generated by gradually increasing the stimulus intensity from 10 to 90 μA. Baseline responses were recorded using half-maximum stimulation intensity at 0.033 Hz. Long-term potentiation (LTP) was induced by theta burst stimulation (TBS) consisting of four theta epochs delivered at 0.1 Hz. In turn, each epoch consisted of 10 trains of four pulses (at 100 Hz) delivered at 5 Hz. The paired-pulse facilitation index was calculated using the equation ((R2-R1)/R1)), where R1 and R2 are the peak amplitudes of the first and second fEPSPs in an interval inter-pulse of 50 ms, respectively. Recordings were filtered at 2.0–3.0 kHz, sampled at 4.0 kHz using an A/D converter, and stored using pClamp10 software (Molecular Devices). Evoked postsynaptic responses were analysed off-line using analysis software (pClampfit, Molecular Devices), which allowed events to be visually detected and which computed only those events that exceeded an arbitrary threshold.

Immunoblotting

The hippocampus of control and fructose-fed degus ($n = 3$ respectively) were randomly dissected on ice and immediately frozen at − 150 °C or processed as previously described [46]. Briefly, the hippocampus tissues were homogenized in RIPA buffer (50 mM Tris-Cl, pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, and 1% sodium dodecyl sulfate [SDS]) supplemented with a protease inhibitor cocktail (P8340, Sigma-Aldrich, Germany) and phosphatase inhibitors (50 mM NaF, 1 mM Na_3VO_4, and 30 μM $Na_4P_2O_7$) using a Potter homogenizer; then, the samples were passed sequentially through different calibre syringes. Protein samples were centrifuged twice at 20.817×*g* at 4 °C for 15 min. The protein concentration was determined using a Bicinchoninic Acid Protein Assay Kit (Pierce Biotechnology, Rockford, IL). Twenty and sixty micrograms of the protein samples were separated by 10% SDS-PAGE and transferred to a polyvinylidene difluoride membrane. The membranes were incubated with primary antibodies (from NeuroMab Facility, Davis, CA: AMPA receptor

subunit 1 [GluA1], vesicular glutamate transporter [VGluT1], AMPA receptor subunit 2 [GluA2]; from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA: phospho-AKT S473 (sc-33437); AKT 1/2/3 (sc-8312), NMDA receptor subunit 2A [GluN2A], synapsin [SYN], synaptophysin [SYP], vesicle-associated membrane protein 1 [VAMP-1, N-methyl-D-aspartate z1 [NMDAz1]; from AbCam, Cambridge, UK: postsynaptic density 95 [PSD-95], 4-hydroxy-2-nonenal [4HNE]; from Cell Signaling Technology: AMPKα (2532); phospho-AMPKα Thr172 (2535)]; from Enzo Life Sciences, Inc., nitrotyrosylated protein [N-Tyr]) and then with anti-mouse, anti-goat, or anti-rabbit IgG peroxidase-conjugated antibodies (Pierce, Rockford, IL), and developed using an enhanced chemiluminescence kit (Western Lightning Plus ECL, PerkinElmer). To analyse the results, all target protein signals were normalized against the loading control (α-tubulin).

Statistical Analysis

All data are presented as the mean ± standard error of the mean (SEM).

Data obtained after the Barnes maze test are expressed as the mean of the four assays of the test phase (see above). In the social interaction and NOR tests, the RI was analysed. Comparisons among treatments were performed with a one-way ANOVA. The assumptions of normally distributed data and homogeneous variances were confirmed using a fitting test on the data. We used nonparametric analyses (Mann–Whitney test) when data could not be transformed to meet these statistical assumptions. Additionally, a repeated-measures ANOVA followed by Tukey's post hoc test was used to analyse the cumulative weight change and daily intake over the course of 28 months, the Barnes maze training data of the different groups and the electrophysiological data. In all analyses $\alpha = 0.05$ was selected to indicate significance. Statistical analyses were performed using the Statistica (StatSoft, Tulsa, OK) software package.

Results

Establishment of MetS-Like Pathogenesis in Degus

Long-Term Fructose Consumption Does Not Alter Body Weight

The effects of long-term fructose consumption on body weight and fasting plasma metabolites are summarized in Table 1. Degus from both groups significantly gained weight with similar rates during the study period (control group: 52.0% and fructose group: 52.5%). At the end of the experiment, fructose-fed degus were slightly heavier, but not statistically different than controls ($F_{1,\ 20} = 0.875$; $P = 0.361$; Table 1). Liver weight and liver to body weight was similar in control and fructose group ($F_{1,\ 18} = 0.939$; $P = 0.345$ and ($F_{1,\ 18} = 0.678$; $P = 0.421$, respectively). Overall, control and fructose-fed degus demonstrated significant differences in cumulative weight change over the course of 28 months ($F_{28,\ 560} = 1.718$; $P = 0.013$; Fig. 1a in the Supplementary data).

Additionally, although the fasting plasma levels of glucose and triglycerides were higher in the fructose-fed animals than in the control degus, these differences were not statistically different (Table 1). The plasma insulin level was 53% higher in the fructose-fed degus than the control degus ($P = 0.014$; Table 1). In addition fructosamine level also was 34% higher in the fructose-fed animal ($P = 0.045$; Table 1). The analysis estimating insulin resistance, as measured using the HOMA index, indicated significant differences between the two experimental groups ($F_{1,\ 19} = 7.802$; $P = 0.012$; see Table 1). Taken together, the elevated fasting plasma insulin and fructosamine levels in addition with HOMA index suggest an insulin-resistant scenario in fructose-fed degus.

Mean daily fructose consumption was higher than the mean daily water intake ($F_{1,\ 20} = 11.42$; $p < 0.01$; Table 1). Overall, control and fructose-fed degus demonstrated significant differences in cumulative daily intake over the course of 28 months ($F_{28,\ 560} = 1.574$; $P = 0.032$; Fig. 1b in the Supplementary data). The average caloric intake was calculated based on daily fluid intakes and the caloric values of fructose (4 kcal/g). Daily caloric intake of fructose-fed degus was 99.47 ± 5.81 kcal/g and accounted for 64% of variation in delta gain weight (final-initial) in fructose group ($p < 0.01$; Fig. 1b).

Long-Term Fructose Consumption Does Not Affect Blood Pressure

There were no significant differences in blood pressure between the groups throughout the experiment (control group: 94.13 ± 6.49 mmHg; fructose group: 87.56 ± 6.17 mmHg; $F_{1,\ 20} = 0.524$; $P = 0.477$; data not shown).

Long-Term Fructose Consumption Influences Glucose Tolerance

The GTT produced a bell-shaped curve in both groups (Fig. 2b). The increase in blood glucose in the fructose-fed animals was more pronounced than that in the control animals, revealing significantly elevated blood glucose levels at 30, 60, and 90 min after the intraperitoneal glucose load compared with those in the control group. As shown in Fig. 1c, blood glucose levels were significantly higher after 30 min in the fructose-fed degus than in the control animals (18.336 ± 0.945 and 15.358 ± 0.834; respectively; $F_{1,\ 13} = 7.677$; $P = 0.015$). After 60 and 90 min, the blood glucose levels began to decrease in both groups, although the levels appeared higher in the fructose-fed group than in the control group (60 min,

Table 1 Body weight, fasting plasma metabolites (glucose, insulin, HOMA index, fructosamine, triglycerides), liver weight, and liver to body weight ratio after the consumption of water or 15% fructose for 28 months

	Dietary group	
	Control group	Fructose group
Body weight		
Body weight 0-time (g)	105.61 ± 5.17	111.69 ± 2.74
Body weight at sacrifice (g)	224.58 ± 6.07	234.97 ± 9.77
Gain weight (Delta)	+116.87 ± 6.91	+123.28 ± 9.17
Glucose (mg dL^{-1})	211.33 ± 16.47	267.56 ± 66.21
Triglycerides (mg dL^{-1})	76.17 ± 10.25	113.56 ± 65.84
Insulin (μU mL^{-1})	0.82 ± 0.10	1.75 ± 0.39*
Fructosamine (μmol L^{-1})	240.76 ± 11.98	363.12 ± 58.66*
HOMA index	411.25 ± 56.77	900.36 ± 176.77*
Liver weight (g)	6.27 ± 0.38	6.95 ± 0.64
Liver to body weight ratio	0.028 ± 0.002	0.031 ± 0.003
Mean daily fructose consumption (g)	19.55 ± 0.84	24.87 ± 1.45*

Values are means ± SEM. Fructose group comprised ten degus whereas control group comprised eleven animals.
*$p < 0.05$ as compared to the control group

13.963 ± 1.463, 7.759 ± 0.832, respectively; $p < 0.01$; 90 min, 7.139 ± 0.814, 5.358 ± 0.244; $P = 0.027$). These deregulations in the capacity to regulate the glucose levels in the fructose-fed animals suggest a clinical profile of insulin resistance.

Long-Term Fructose Consumption Increases BMR

No significant differences in body weight between the control degus (time 1: 187.67 ± 5.84 g and time 2: 223.29 ± 6.29 g)

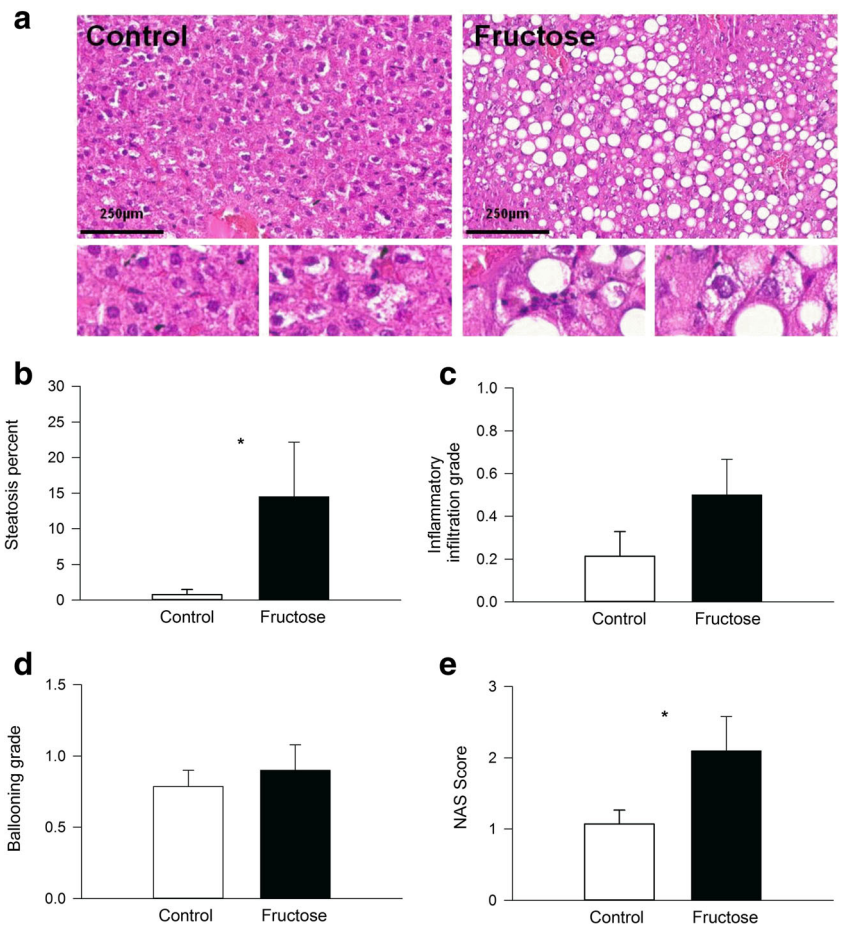

Fig. 2 Effect of chronic fructose consumption induced changes similar to those observed in human NAFLD (**a**) representative histological images (with haematoxylin and eosin staining) from the control and fructose-fed groups (**b**) steatosis percent (**c**) inflammatory infiltration grade (**d**) hepatocellular hypertrophy or "ballooning" grade (**e**) NAS system. Values are shown as the mean ± SEM. The data were analysed statistically using one-way ANOVA followed by Tukey's post hoc test. *Asterisk* indicates significant differences between both groups. *$p < 0.05$; **$p < 0.01$ ($n = 12$ animals for control group and 10 animals for fructose-fed degus)

and the fructose-fed degus (time 1: 199.59 ± 8.73 g and time 2: 216.58 ± 7.42 g) were found at the time that BMR were measured ($F_{1,\ 18} = 1.289$; $P = 0.271$ and $F_{1,\ 17} = 0.499$; $P = 0.499$, respectively). Nevertheless, we observed that BMR at time 1 was 11% higher in the fructose-fed group (158.05 ± 7.01 mLO_2h^{-1}) in comparison to control degus (140.48 ± 4.96 mLO_2h^{-1}, $F_{1,\ 18} = 4.181$; $P = 0.056$; Fig. 1c) and 20% higher in the fructose-fed group (181.50 ± 7.96 mLO_2h^{-1}) than in the control group at time 2 (146.04 ± 9.45 mLO_2h^{-1}, $F_{1,\ 17} = 7.406$; $P = 0.014$; Fig. 1d).

Long-Term Fructose Consumption Is Associated with NAFLD

The histological analysis of the liver samples showed that long-term fructose consumption induced changes similar to those observed in human NAFLD, including liver steatosis, hepatocyte ballooning, and inflammatory infiltration (Fig. 2a). The steatosis percentage was 18-fold higher in the fructose-fed group than in the control group (14.50 ± 7.69% and 0.786 ± 0.712%, respectively; $P = 0.046$; Fig. 2b). Although more inflammatory foci were observed in the fructose-fed group than in the control group, this difference was not significant (Fig. 2c). Additionally, the number of "ballooned" hepatocytes was similar between the two groups (Fig. 2d). Importantly, the NAS of the fructose-fed animals was two-fold higher in that of the control degus ($P = 0.038$; Fig. 2e). Finally, the Sirius staining analysis for determining the grade of fibrosis did not show differences between the two groups (data not shown).

Long-Term Fructose Consumption Increases Oxidative Stress

To determine whether long-term fructose consumption affects redox balance, we measured the levels of 4HNE, one of the final products of lipid peroxidation, and the levels of nitrosylated protein (N-Tyr), which are products of protein oxidation. We observed that both proteins displayed significant increasing trends in the fructose-fed degus compared with the control animals (N-Tyr, $F_{1,\ 4} = 15.94$; $P = 0.016$; 4HNE, $F_{1,\ 4} = 20.85$; $P = 0.010$; Fig. 3a, b). These results indicate that long-term fructose consumption induces several oxidative stress-related events, triggering lipid peroxidation and protein nitrosylation in the hippocampus of degus. In addition, we also measured the levels of the activity of two metabolic proteins: phospho-AKT Ser473and phospho-AMPK. Our results revealed that long-term fructose consumption caused impairment of hippocampal insulin signalling based on a significant reduction in phospho-Akt ($F_{1,\ 4} = 17.89$; $P = 0.013$; Fig. 2a, b in the Supplementary data). Moreover, this decrease in fructose-fed animals represents 51% when compared with control group. The phosphorylated AMPK level was unchanged between control and fructose-fed group ($F_{1,\ 4} = 0.006$; $P = 0.941$; Fig. 2a, c in the Supplementary data).

Long-Term Fructose Consumption Impairs Hippocampus-Dependent Cognitive Performance in Degus

The degus were subjected to several behavioural tasks to investigate the possible role of long-term fructose consumption on cognition.

Open Field Test

To evaluate the general state of the animals, we performed the open field test. In this context, no significant differences were found between the groups (all $p > 0.05$), suggesting that the degus exhibited normal general behaviour (Fig. 4a–c).

Social Interaction Test

The analysis of the effects of fructose consumption on the performance of the degus in the social interaction test indicated a significant difference between the two groups in both session 1 ($F_{1,\ 20} = 9.203$; $p < 0.01$) and session 2 ($F_{1,\ 20} = 42.50$; $p < 0.01$), taking the RI as the dependent variable (Fig. 5a). During session 1, both groups showed a clear preference for the compartment with partner I. The control and fructose-fed animals spent 80 and 70% more time, respectively, in the compartment with the unfamiliar degu than in the compartment with the empty cup (both $p < 0.01$; Fig. 5b), although no significant differences were found between the groups. During session 2, the control degus showed a clear preference for the compartment containing the novel unfamiliar degu (partner II). They spent 36% more time in the compartment with the new partner than in the compartment with the familiar partner ($p < 0.01$; Fig. 5b). By contrast, no such preference was detected in the fructose-fed animals, which spent roughly equal amounts of time in the two compartments, demonstrated decreased social memory compared with the control degus (Fig. 5b).

NOR Test

Then we studied the effect of the fructose diet on NLR/NOR, which is a double test used to evaluate cognition, particularly recognition memory. Taking the RI as the dependent variable, the analysis of the effects of fructose consumption measured across the NLR trial revealed a significant difference between the two groups ($F_{1,\ 20} = 47.72$; $p < 0.01$; Fig. 6a). The control degus spent twice as much time interacting with the moved object than with the familiar object ($p < 0.01$, Fig. 6b). By contrast, the fructose-fed degus spent equal amounts of time with the objects and made equally frequent visits to each object ($P = 0.399$; Fig. 6b), suggesting that fructose consumption impairs spatial working memory in degus fed fructose. Similarly, during the NOR assay, we observed a significant difference between the two groups ($F_{1,\ 20} = 18.86$; $p < 0.01$; Fig. 6a). In this trial, the

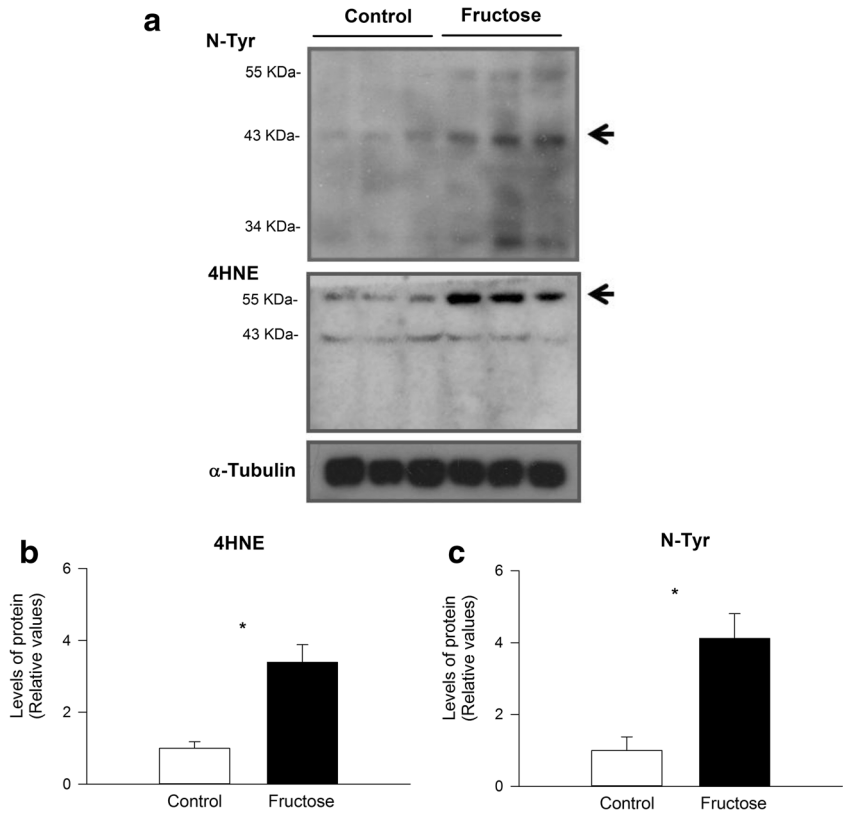

Fig. 3 Biochemical analysis of oxidative stress-related proteins in the hippocampus of control degus and fructose-fed group (**a**) western blot analysis of 4HNE and N-Tyr (**b**) densitometric analysis of 4HNE (**c**) densitometric analysis of N-Tyr. The data were analysed statistically using one-way ANOVA followed by Tukey's post hoc test. *Asterisk* indicates significant differences between both groups. *$p < 0.05$; **$p < 0.01$. Each bar corresponds to data from a single group, represented as the mean ± SEM ($n = 3$)

control degus showed a clear preference for the novel object. They spent four-fold more time interacting with the novel object than with the familiar object ($p < 0.01$, Fig. 6b). Again, no such preference was detectable in the fructose-fed degus, which spent almost equal amounts of time in the two compartments ($P = 0.942$; Fig. 6b), suggesting decreased memory and predilection for novel experiences compared with the control group.

Barnes Maze Test

The Barnes maze test indicated a significant difference between the two groups ($F_{1, 180} = 23.239$; $p < 0.01$) and in the time to find the escape hole ($F_{9, 180} = 2.041$; $P = 0.037$) but not in interaction group × time values ($F_{20, 180} = 1.516$; $P = 0.145$) regarding the latency to the first visit of the escape hole (Fig. 7a). After the second day of the Barnes maze training, the control animals needed significantly less time than the fructose-fed degus to find the escape hole. More importantly, during the testing phase of the Barnes maze assay, we found a significant effect on the time to the first visit of the escape hole ($F_{1, 19} = 10.66$; $p < 0.01$, Fig. 7b, d). The control degus located the escape hole eight-fold faster than the fructose-fed degus (Fig. 7b). When the maze was split into four zones, we found that the control and fructose-fed degus expressed similar spatial preferences for the target area ($F_{1, 19} = 4.804$; $P = 0.041$, Fig. 7c). The analyses of the reference memory errors showed

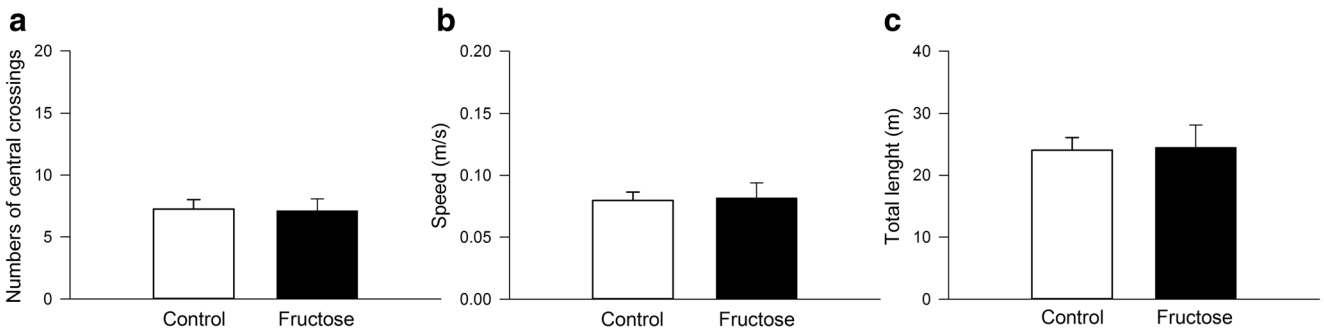

Fig. 4 Exploration behaviour in the open field arena of control degus and degus fed 15% fructose ($n = 12$ animals for control group and 10 animals for fructose-fed degus) (**a**) number of central crossings (**b**) average speed (**c**) total distance travelled on the open field arena during 5 min of testing. The data were analysed statistically using one-way ANOVA followed by Tukey's post hoc test. No *Asterisk* over the bars indicates non-significant differences ($p > 0.05$)

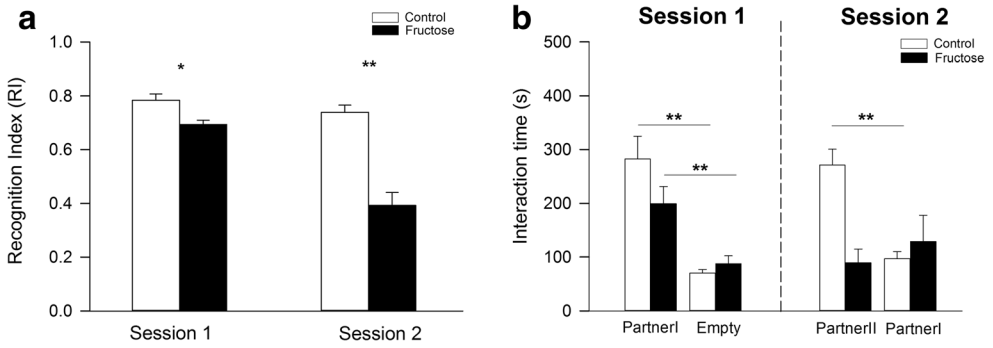

Fig. 5 Evaluation of the cognitive performance of control and fructose-fed groups using social interaction rest ($n = 12$ animals for control group and 10 animals for fructose-fed degus) (**a**) RI for session 1 (social affiliation and sociability, expressed as the quotient of the time the subject degu spent with partner I divided by the sum of the time spent with partner I and the empty cup) and RI for session 2 (social memory and novelty, expressed as the quotient of the time the subject degu spent with partner II divided by the sum of the time spent with partners I and II) (**b**) average interaction time for partner 1 vs. the empty cup during session 1 and average interaction time for partner II vs. the familiar partner during session 2. The results are expressed as the mean ± SEM. The data were analysed statistically using one-way ANOVA followed by Tukey's post hoc test. *Asterisk* indicates significant differences between both groups. $*p < 0.05$; $**p < 0.01$

that the control degus committed fewer reference memory and working memory errors than did the fructose-fed animals. As shown in Fig. 2a in the Supplementary Data, we detected significant differences between the two groups ($F_{1,\ 19} = 5.772$; $P = 0.027$) in the number of reference memory errors. Similarly, the analysis of the working memory errors showed a significant difference between the two groups ($F_{1,\ 19} = 6.778$; $P = 0.017$; Fig. 2b in the Supplementary Data).

Long-Term Fructose Consumption Impairs Synaptic Plasticity in the CA3-CA1 Synapse of the Degu Hippocampus

Basal synaptic transmission was assessed by the I/O relationship, in which the fEPSP slope is obtained in response to increasing electrical stimulation in the CA1 area of the stratum radiatum of the hippocampus to assess dendritic activation. As Fig. 8a shows, the fructose treatment did not change the magnitudes of the fEPSP slopes. The repeated-measures ANOVA results of the baseline I/O functions confirmed that fEPSP slope (Fig. 8b) increased as a function of stimulus intensity ($F_{8,\ 24} = 60.288$; $p < 0.01$). However, no significant differences were found between the two groups ($F_{1,\ 24} = 0.040$; $P = 0.853$) or their interaction treatment × stimulus intensity values ($F_{8,\ 24} = 0.794$; $P = 0.613$). The paired-pulse facilitation (PPF) index was assessed by the ratio between two pulses separated by 50 ms. The fructose treatment generated an impairment in the PPF, as at this interval, the fructose treatment reduces the facilitation of the second pulse compared with that in the controls ($F_{1,\ 10} = 6.843$; $P = 0.026$). We next assessed whether synaptic plasticity was affected at the CA3–CA1 synapses of the fructose-treated animals using LTP as a representation of synaptic strength. In the control animals, the delivery of TBS induced a persistent increase (60 min) in the fEPSP slope, as expected in young-adult control degus (Fig. 8c, white dots and bars). By contrast, in the

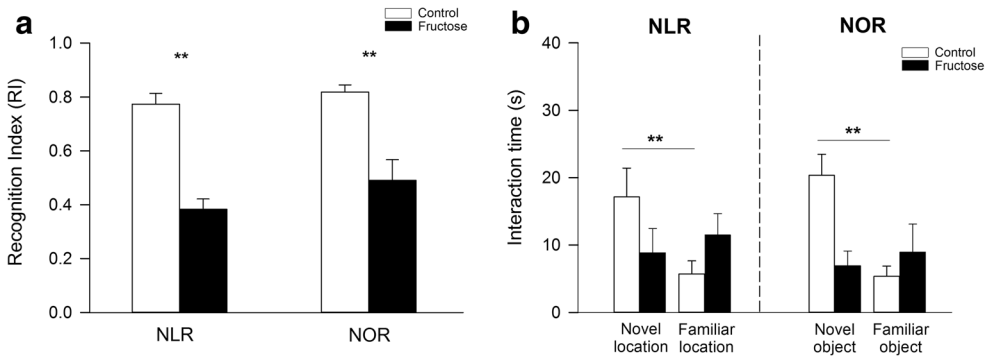

Fig. 6 Evaluation of the cognitive performance of control and fructose-fed groups using novel object/novel local recognition ($n = 12$ animals for control group and 10 animals for fructose-fed degus) (**a**) analysis of the RI for the "NLR" trial (time spent exploring the novel object location/time spent exploring the novel and familiar object locations) and analysis of the RI for the "NOR" trial (time spent exploring the novel object/time spent exploring the novel and familiar objects) (**b**) average exploration time for the novel and familiar object locations during the NLR trial and average exploration time for the novel and familiar objects during the NOR trial. The results are expressed as the mean ± SEM. The data were analysed statistically using one-way ANOVA followed by Tukey's post hoc test. *Asterisk* indicates significant differences between both groups. $*p < 0.05$; $**p < 0.01$

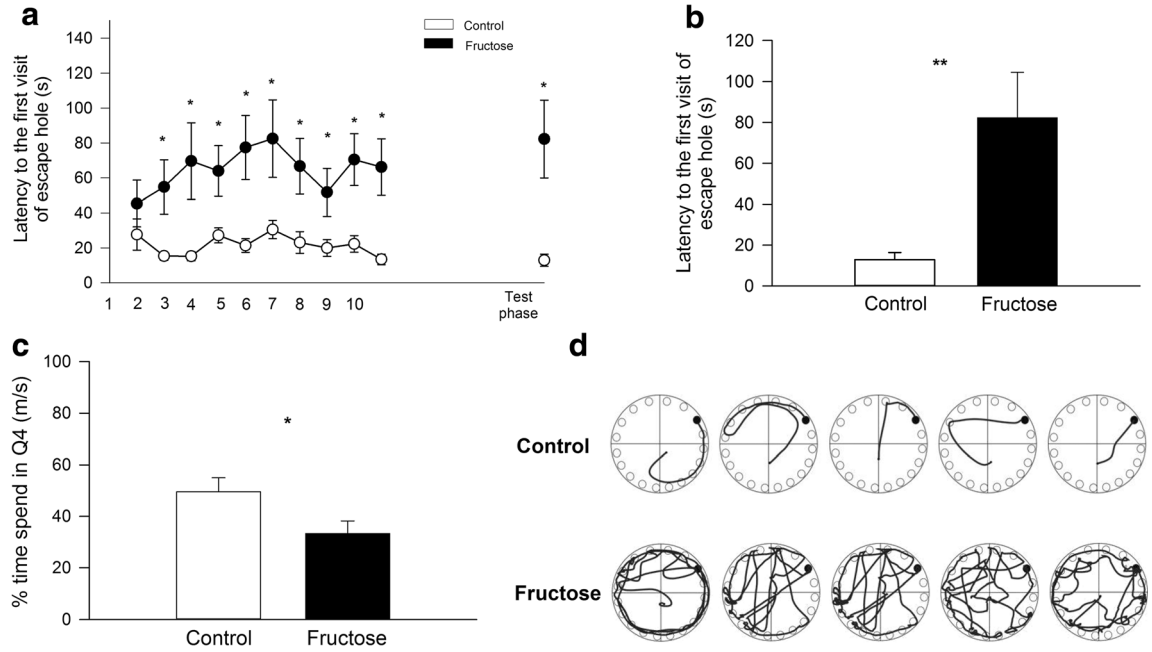

Fig. 7 Evaluation of the cognitive performance of control and fructose-fed groups using Barnes maze test ($n = 12$ animals for control group and 10 animals for fructose-fed degus) (**a**) learning curve of the latency of the first visit to the escape hole for the 10-day training session and the testing phase (**b**) latency to the first visit of the escape hole for the testing phase (**c**) percentage of time spent in the quadrant with the escape hole for the testing phase (**d**) paths taken by representative animals (e.g. close to the group mean) for the latency to the first visit of the escape hole of the control and fructose-fed groups over 28 months. The grey area represents the quadrant of the escape hole. The escape hole is indicated in black. The results are expressed as the mean ± SEM. The data were analysed statistically using one-way ANOVA followed by Tukey's post hoc test. *Asterisk* indicates significant differences between both groups. $*p < 0.05$; $**p < 0.01$

fructose-fed group, the LTP magnitude was significantly reduced, suggesting an impairment in the synaptic plasticity of those animals ($F_{1,9} = 5.285$; $P = 0.047$; Fig. 8d).

Long-Term Fructose Consumption Alters Synaptic Proteins

To observe the composition of the synapses, we performed a Western blot analysis of the pre and postsynaptic proteins in the degu hippocampus. No consistent differences were observed in the presynaptic proteins (Fig. 9a, b). Furthermore, no differences were observed in the SYP, SYN, and VAMP-1 levels between the two groups (Fig. 9b). In the case of VGluT1 protein, a significant decrease was observed in the fructose-fed group ($F_{1,4} = 8.253$; $P = 0.045$) compared with the control group (Fig. 9b). For postsynaptic proteins, we observed clear decreases in the PSD-95 and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor GluA1 levels in the fructose-fed group ($F_{1,4} = 34.91$; $p < 0.01$ and $F_{1,4} = 24.34$; $p < 0.01$, respectively) compared with the control group (Fig. 9c), whereas in the case of the AMPA receptor GluA2 and the NMDA receptor GluN2A, no changes were detected between the two groups (Fig. 9d). These results indicate decreases in some postsynaptic rather than presynaptic hippocampal proteins in degus consuming 15% amount of fructose for 28 months.

Discussion

The purpose of this study was to investigate the consequences of long-term fructose consumption on the normal ageing process in a long-living animal model rodent, able to resemble some of the pathophysiological processed of human age-related disorders. We choose degus as our rodent animal model because this species can live for several years in laboratory condition, so we can have better understand of the metabolic effects of prolonged consuming fructose. We measured a set of clinical criteria for a condition similar to MetS. We also extended our analysis to evaluate the effects of metabolic dysfunctions on brain function, impacting synaptic plasticity and cognitive performance.

MetS refers to a cluster of cardiometabolic risk factors (i.e. obesity, insulin resistance, impaired glucose tolerance, hypertension, and dyslipidemia), associated to an increased risk of developing atherosclerotic cardiovascular disease and diabetes [9, 47]. However, there are discrepancies regarding which of these factors is the most commonly blamed for the development of MetS [48]. Another parameter related to MetS that has recently gained importance is NAFLD [49, 50]. NAFLD appears to be associated with insulin resistance and closely linked to Mets and type 2 diabetes [9, 51], in fact, NAFLD represents the hepatic manifestation of MetS [48, 52]. The results of the present study have showed that degus fed with

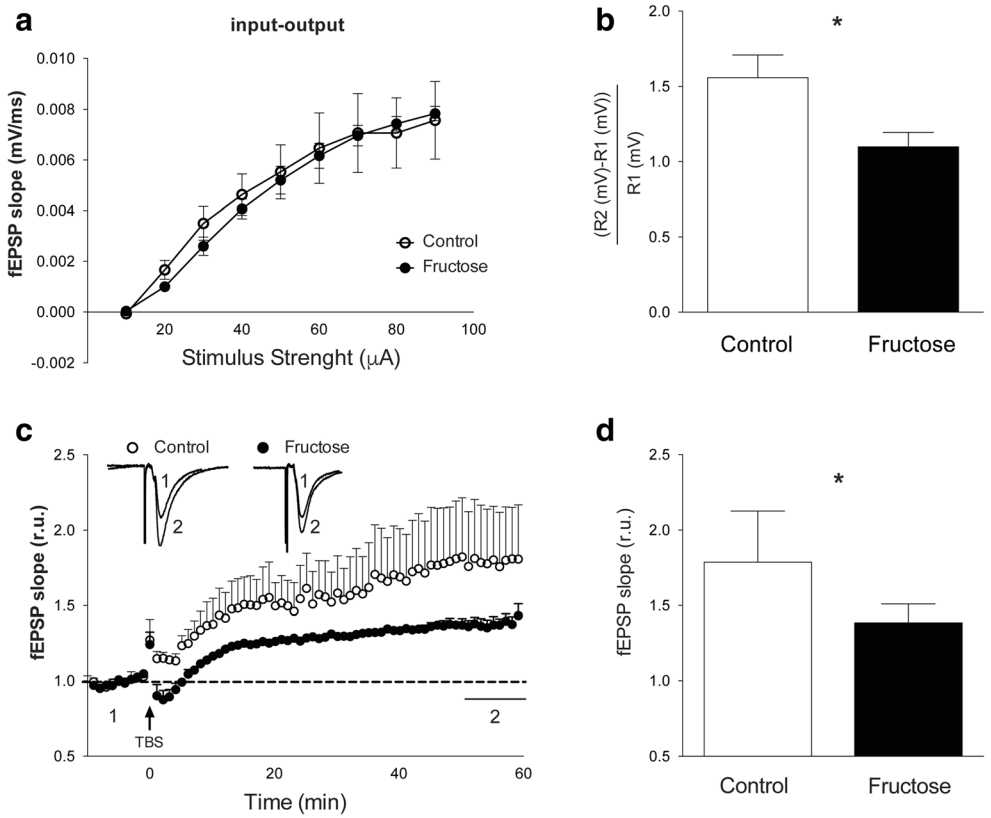

Fig. 8 In vitro electrophysiological recordings of control and fructose-fed degus (**a**) input-output curves for fructose-fed and control degus (**b**) plot of the PPF measured as R2 (mV) – R1 (mV) / R1 (mV) between the fructose-fed and control groups (**c**) time curve of the LTP experiment in the fructose-fed and control groups. A TBS was delivered after 10 min of stable baseline recording. Inset: representative fEPSP traces before (1) and after (2) the TBS for each experimental group (**d**) averaged fEPSP magnitude during the last 10 min of the LTP experiment for the fructose-fed and control groups. Bars represent the mean ± SEM. The data were analysed statistically using repeated-measures ANOVA for the baseline I/O functions and one-way ANOVA followed by Tukey's post hoc test for the rest ones. *Asterisk* indicates significant differences between both groups. $*p < 0.05$. Two to three hippocampal slices were used per animal, and $n = 3$ animals were used per group

15% of fructose over a prolonged intake (28 months) have developed several MetS-like condition features.

Long-Term Fructose Consumption Induces Metabolic Derangements in *Octodon degus*

Body weight gain has been recognized as one of the main visible harmful effects of excessive fructose consumption in several studies performed in animal models and humans subjects [3, 5, 14]. In our study, the fructose daily energy consumption was able to explain 64% of variation in body weight (Fig. 1a), however, the ad libitum access to fructose solution over 28 months did not lead to increased weight gain. In accordance with our result, there are other studies that have failed to show the effect of fructose as a weight-increase promoting sugar [13, 16, 18, 23]. A possible explication of why we did not found differences in body weight will be explained more in detail during the explanation of BMR results (see below).

Previous reports have shown that high dietary fructose levels increase blood parameters, such as plasma triglycerides and glucose concentrations, along with liver mass [53, 54]. However, in our study, 28 months of fructose intake induced moderate but not significant increases in fasting plasma triglycerides and glucose compared with those of the control group. Similar results were reported by Levi & Werman [16], and Tillman et al. [18] in terms of fasting plasma glucose and Reiser et al. [55], and Tillman et al. [18] in terms of serum triglycerides. However, plasma insulin and fructosamine concentrations in the fructose-fed degus were significantly greater than in the control animals (Table 1). In fact, fructose-fed degus had 53% higher plasma insulin and 34% plasma fructosamine than controls (Table 1). Because, fructosamine reflected glucose levels over a recent period (about 2–3 weeks), together these results can confirm the results of previous studies demonstrating that fructose consumption leads to glucose intolerance and decrease insulin sensitivity [56]. More importantly, we found that the HOMA index and GTTs of the fructose-fed group were significantly higher than

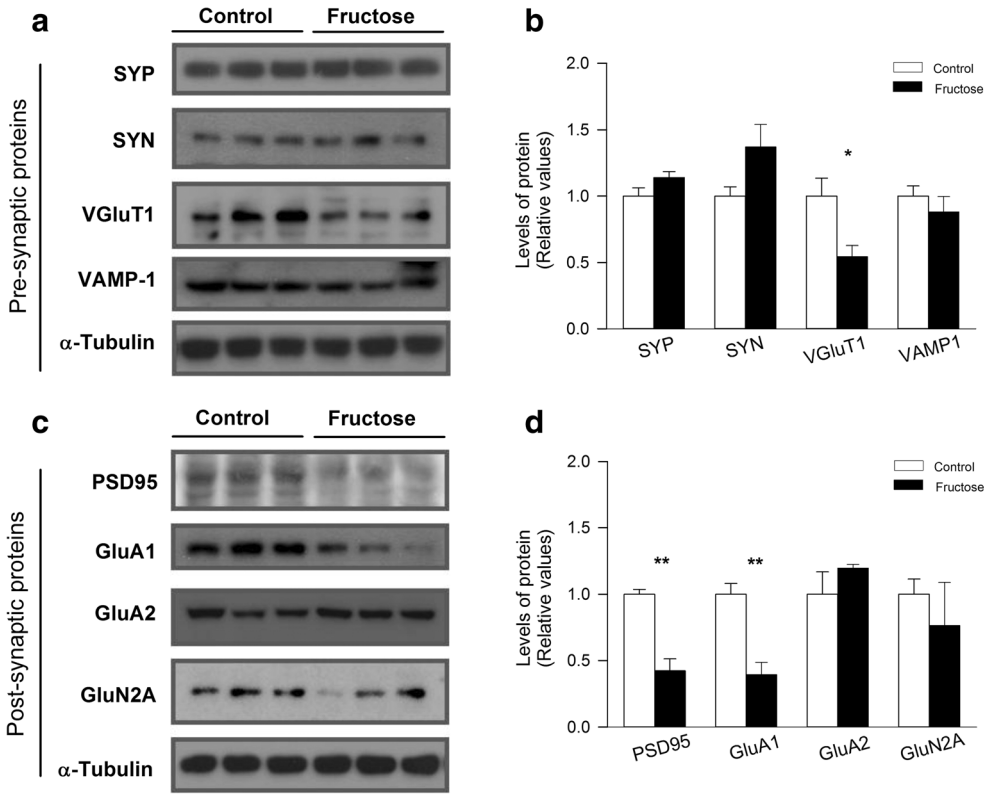

Fig. 9 Biochemical analysis of hippocampal presynaptic and postsynaptic proteins (**a**) western blot analysis of presynaptic proteins (**b**) densitometric analysis of SYP, SYN, VGluT1, and VAMP-1 proteins (**c**) western blot analysis of postsynaptic proteins (**d**) densitometric analysis of PSD-95, GluA1, GluA2, and GluN2A proteins. The data were analysed statistically using one-way ANOVA followed by Tukey's post hoc test. *Asterisk* indicates significant differences between both groups. $*p < 0.05$; $**p < 0.01$. Each bar corresponds to data from a single group, represented as the mean ± SEM (n = 3 animals per group)

the control group (Table 1 and Fig. 1a, respectively), which further confirmed the insulin-resistance state associated with the fructose diet.

While evidence of chronic high-fructose induced hypertension, tachycardia, and cardiac hypertrophy has been documented in several studies using animal models [23, 57, 58]. The current study we did not find significant effect of fructose intake on the systolic blood pressure in the fructose-fed degus. These findings are consistent with previous results in animal models and human subjects [59–61]. Further research will be needed to determine if other cardiac functions could be affected (e.g. diastolic arterial pressure).

In humans and non-human animals, the liver is the main organ involved in the metabolism of fructose and glucose. When liver glycogen is replenished, fructose intermediates are directed towards de novo triglyceride synthesis. In this way, metabolically speaking, the liver-related consequences of a high-fructose diet will be similar to those of a high-fat diet [13, 62]. Thus, these are the reasons why fructose has been identified as a key player in MetS [5, 63]. In fact, currently studies emphasize the role of high fructose intake in insulin resistance, oxidative stress, and subsequent lipid peroxidation, as well as proinflammatory cytokine, adipokine and mitochondrial dysfunction, which favour the development and progression of NAFLD in humans and rodents [64, 65]. We measured several criteria for NAFLD, such as steatosis, inflammatory infiltration, and hepatocellular hypertrophy or "ballooning" grades, and we found higher levels of steatosis in the fructose-fed animals, which indicated that they exhibited NAFLD. In addition to the supporting epidemiological data, the pathogenesis of NAFLD has been related to deregulated energy metabolism (see below). For example, Müller et al. [66] reported elevated resting energy expenditure in patients with liver cirrhosis. Similarly, Tarantino et al. [67] registered increased energy expenditure in male morbidly obese NAFLD patients with MetS, whereas insulin resistance, cholesterol, and HOMA index did not change compared with patients without MetS. In this context, increased BMR may be a clue of MetS [67].

Because the major components contributing to the systemic energetic homeostasis are energy intake and expenditure [68], to identify potential contributors to no increase in body weight, despite an increase in fructose intake, we further measured energy expenditure by evaluating BMR. The BMR of the fructose-fed group was 11 and 20% higher than that of the control group (Fig. 2b). In this regard, previous studies in animal model fed with high-caloric diet or fructose feeding showed a reduction in body weight which was accounted by anallometric increased energy expenditure and a reduced adipose mass despite increases in food intake [18, 68]. The high basal energy expenditure in the fructose-fed group probably can be explained in terms of the flexibility of metabolic routes in response to a decrease in energy storage due to an increase in the mobilization of metabolic fuel allocated to growth [69,

70]. In our experiment, we performed the first long-term study that replicates human sugar consumption behaviour over time, from pup to adult degus. Four-month-old degus were directly exposed to 15% fructose consumption. In those studies to examine the physiological changes induced by fructose consumption that have utilized mice or rats, they have done so using adult animals. In this context, elevated metabolic rate during maturation probably was able to fully oxidize the fructose [18]. In addition, the increased energy expenditure in the fructose-fed group could explain why we did not find a significant increase in body fat mass (data not shown). Moreover, this elevated metabolism may also carry a cost in terms of increased mitochondrial production of reactive oxygen species (ROS), triggering oxidative damage to lipids and proteins [71, 72; see below].

Taken together, the different symptoms exhibited by the fructose-fed degus, i.e. insulin resistance with increased insulin and fructosamine plasma concentrations, impaired glucose tolerance, a high HOMA index, a high BMR, and a NAFLD diagnosis, are components of MetS-like condition. A reasonable hypothesis above the fact degus is unable to present a complete MetS picture could be the influence of protective mechanisms age-depended in degus. However, further detailed investigations will be necessary.

Fructose-Induced MetS-Like Condition Affects Brain Function

Although the peripheral effects of MetS-like condition have been characterized, several deleterious types of MetS-related signalling have been observed to reach the brain, revealing several effects of this disease on cognition functions and emotion [12]. It is known that fructose is preferentially metabolized by the liver whereas glucose is preferentially used by the brain, GLUT5 is the only glucose transporter specific for fructose with no ability to transport any other sugar as glucose or galactose and it has been recently demonstrated to be expressed by hippocampal cells, which corroborate that fructose is able to be consumed by the brain [12, 73]. Fructose appears more frequently in an active form and has a higher reactivity than glucose. In addition, when fructose intake by neurons increases, several alterations occur, including the following: (a) free radical production by passes the neutralizing mechanisms and the redox balance in neurons becomes impaired; (b) the production of advanced glycation-related products increases; (c) glycoxidation processes increases; and (d) oxidative stress events increases. All of these events are related to mitochondrial dysfunction, lipid peroxidation, and post-translational alterations of proteins [74, 75]. In fact, despite other studies mention that fructose-induced cognitive impairment was ascribed to the concomitant insulin resistance, recent studies have specifically described neurotoxicity induced by the peroxidation of lipids and the oxygen-related modification of proteins related to the intake of this carbohydrate, triggering reduced acetylcholinesterase activity and altered mitochondrial function, which are crucial for supporting brain function and plasticity [15, 76, 77]. Recent studies have found altered activities of mitochondrial respiratory complexes in hippocampus of mice exposed to high fructose intake that may contributed to early modification and lead to the ROS accumulation [73]. We observed that the levels of both 4HNE, a final product of lipid peroxidation, and N-Tyr, a product of proteinoxidation processes, were increased in the hippocampus of the fructose-fed animals. These results indicate that fructose-induced MetS-like condition affects several oxidative stress events in the brain, triggering lipid peroxidation and protein nitrosylation, which is in accordance with previous studies showing similar results in both the cerebral cortex and hippocampus, whereby acute fructose administration induced oxidative damage to lipids and proteins, enzyme antioxidant defence alterations and cognitive impairment associated with memory retention [12, 76]. Moreover, the long-term fructose consumption decreased insulin-stimulated Akt phosphorylation to 51% of control level (Fig. 2b in the Supplementary data). Surprisingly, hepatic AMPK phosphorylation resulted in no changes in fructose-fed group as compared to control group. This is in line with previous studies [78–81], and point out to a specific effect of long-term fructose consumption on hippocampal insulin signalling in this long-lived animal model.

Fructose-Induced MetS-Like Condition Impairs Spatial Learning and Memory in *Octodon degus*

Because another clinical manifestation of sugar-induced MetS-like condition is a decline in learning and memory performance in both animal models and humans subjects [12, 14, 82], we evaluated whether long-term fructose consumption affected these activities in our animal model. To study the general behaviour of degus, we performed the open field test which showed no differences between the groups, suggesting normal general behaviour and motor skills (Fig. 4a–c). Degus are highly social rodents with complex social behaviours [31], wherein the ability to learn and remember individuals is critical for the stability of social groups [83]. As such, we performed a social interaction test, which is a double test used to evaluate both social bonding and social memory. We also used the NOR test to evaluate cognition, particularly recognition memory. Finally, we used the Barnes maze test to study spatial learning and memory, processes that both depend, in part, on hippocampal structure [44, 84].

In the social interaction test, we observed that the control and fructose-fed degus spent similar amounts of time exploring the new partner during session 1 (Fig. 5b). Interestingly, degus in both groups spent more time interacting with the new animal than the inanimate object. In this sense, it is relevant that the levels of tryptophan, the essential amino acid that acts as a natural mood regulator, did not change in male rats after

the long-term consumption of a sugar-rich diet [70, 85]. However, when we used the RI [86], we observed significant differences between the two groups, suggesting initiation of the process of losing social bonds (Fig. 5a). More importantly, during session 2, there was a clear difference between the two groups, with the fructose-fed degus spending more time with the familiar partner than the new partner compared with the control degus, suggesting that fructose consumption impairs social recognition in these animals (Fig. 5b). Using the RI, we observed a significant decrease in this variable in the fructose-fed degus, indicating a reduction in social memory for novel experiences (session 2, Fig. 5b).

In the NOR/NLR tests, we observed decreases in the spatial working memory of the fructose-fed degus compared with the control degus. The control degus spent more time exploring the novel object and novel object location than the familiar ones, whereas the fructose-fed animals spent equal amounts of time with both objects in each case (Fig. 6b). For both the NLR and NOR sessions (Fig. 6a), we observed a significant decrease in the RI of the fructose-fed degus, a result that confirms a loss of recognition memory [87].

In the Barnes maze test, we observed a significantly increased latency time for finding the escape hole in the fructose-fed group compared with the control group. In fact, the fructose-fed animals required eight-fold more time to find the escape hole than did the control animals (Fig. 7b–d). Taken together, these behavioural data suggest that fructose-induced MetS-like condition may therefore impair hippocampal-dependent and independent memory this animal model.

Fructose-Induced MetS-Like Condition Reduces Functional and Structural Synaptic Plasticity in *Octodon degus*

Because behavioural analyses alone may not be able to determine the mechanisms underlying the observed cognitive impairments associated with fructose consumption, we also performed a series of functional and biochemical analyses. The metabolic-associated memory deficit likely involves altered information processing through hippocampal circuits because we observed a disruption in declarative and spatial-related memory, which are known to critically depend on hippocampal circuitry [88]. Neuronal communication requires a high amount of energy and is critically related to the cellular metabolism and energy supply, both dependent of the glucose availability [89]. An imbalance in the glucose metabolism affects neuronal circuits and is especially relevant for hippocampal functioning because this brain structure has a high density of excitatory synapses, which activity strictly dependent on glucose availability [90]. The hippocampus processes and stores information via synaptic plasticity mechanisms, such as LTP, which is an electrophysiological manifestation of changes in synaptic strength and improvements in synaptic connectivity [91]. In line with the deteriorations in behaviours presented above, we observed impaired synaptic plasticity in the fructose-fed degus, as demonstrated by a decreased LTP magnitude compared with that of the control degus. This finding was similar to that observed in mice fed fructose for 7 weeks [14]. Additionally, we observed a change in the PPF index, which points to impaired transmitter release of the presynaptic compartment [92, 93]. In hippocampal neurons, the release probability is low, and for that reason, the first spike will cause a small fEPSP, but the build-up of calcium in the presynaptic terminal will lead to an increased release probability on the second spike, resulting in greater transmitter release, a greater postsynaptic response, and a higher PPF. The reduced PPF index generated by fructose consumption suggests an increase in the release probability because both spikes became more similar, indicating that the first pulse will deplete the available transmitters, causing the second pulse to release fewer transmitters [92, 93]. By contrast, the basal synaptic transmission was not affected by the fructose treatment, as revealed by the I/O relationship, which suggests that the alterations observed in the plasticity process are not related to a synaptic loss but could involve modifications in the levels of different synaptic proteins related to LTP induction and consolidation. In accordance with the reduction in the PPF index and the LTP process, we observed that the chronic consumption of 15% fructose decreased the levels of the presynaptic protein VGluT1 and the postsynaptic proteins PSD-95, and GluA1, which are involved in synaptic plasticity events as LTP and learning process, specifically in synaptic vesicles release, scaffolding of receptor, and synaptic transmission, respectively [94, 95].

Together, these results indicate that fructose-induced MetS-like condition reduces synaptic protein levels, inducing synaptic function impairments and triggering learning and memory performance deficits in this long-lived animal model.

Conclusions

High intake food-induced metabolic disorders, such as MetS, disrupt functional and cognitive brain processes. Using a different model, we have previously demonstrated the detrimental effects of high fructose consumption on neural activity. In the present study, for the first time, we show that long-term fructose consumption affects normal ageing process by means of induction of MetS-like conditions altering ultimately, synaptic plasticity and cognition. Our results show that fructose-fed male degus exhibited several hallmarks of MetS, such insulin resistance with increased insulin and fructosamine plasma concentrations, a high HOMA index, impaired glucose tolerance and high metabolism. More importantly, our results revealed higher levels of steatosis in the fructose-fed animals, indicating that they exhibited NAFLD. Using the

degu, a long-lived animal, not only enabled us to perform the first long-term study that replicates human sugar consumption behaviour over time but also led us to understand the mechanisms by which metabolic disorders could affect cognitive abilities. Moreover, in addition to systemic metabolic alterations, excessive fructose intake also induced several neuropathological events, such as oxidative stress processes, causing hippocampal lipid peroxidation and protein nitrosylation, together with synaptic protein loss. These changes might be responsible for the alterations in synaptic plasticity and transmitter release observed in these cognitively impaired animals. Considering that this rodent species has been proposed as a potential "natural" reliable model of human age-related cognitive impairment disorders, our results show that a long-term fructose diet triggers several facets of MetS that eventually cause brain disorders, in particular, synaptic dysfunction and reduced cognition; whether such trends also occur in humans requires further research. If well our data fail to support for fructose intake in altering another MetS parameters such body weight gain, circulating glucose, triglycerides, increased adiposity, and systolic hypertension in degus. Further research will be necessary to understand if these animal model present protective mechanisms linked to youth, or associated with gender differences or higher doses of fructose may accentuate this current result.

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Compliance with Ethical Standards

Housing, treatment and euthanasia of animals met the guidelines set by the National Institutes of Health (NIH, Baltimore, MD, USA). All experimental procedures involving animals were approved by the Bioethical and Biosafety Committee of the Faculty of Biological Sciences of the Pontificia Universidad Católica de Chile (CBB-121-2013). All efforts were made to minimize animal suffering and to reduce the number of animals used.

Conflict of Interest The authors declare that they have no conflict of interest.

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