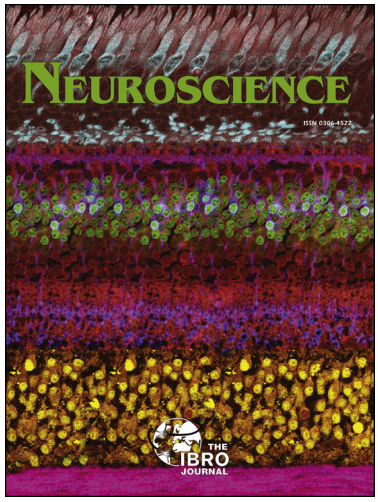


Spatial memory impairments in a prediabetic rat model

Edna Soares, Rui D. Prediger, Sara Nunes, Adalberto A. Castro, Sofia D. Viana, Cristina Lemos, Carolina M. De Souza, Paula M. Agostinho, Rodrigo A. Cunha, Eugénia Carvalho, Carlos A. Fontes Ribeiro, Flávio Reis, Frederico C. Pereira

PII: S0306-4522(13)00649-0
DOI: http://dx.doi.org/10.1016/j.neuroscience.2013.07.055
Reference: NSC 14783

To appear in: *Neuroscience*

Accepted Date: 18 July 2013

Please cite this article as: E. Soares, R.D. Prediger, S. Nunes, A.A. Castro, S.D. Viana, C. Lemos, C.M. De Souza, P.M. Agostinho, R.A. Cunha, E. Carvalho, C.A. Fontes Ribeiro, F. Reis, F.C. Pereira, Spatial memory impairments in a prediabetic rat model, *Neuroscience* (2013), doi: http://dx.doi.org/10.1016/j.neuroscience.2013.07.055

Spatial memory impairments in a prediabetic rat model

Edna Soares [1], Rui D. Prediger[2], Sara Nunes[1], Adalberto A. Castro[2], Sofia D. Viana[1], Cristina Lemos[3], Carolina M. De Souza[3], Paula M. Agostinho[3], Rodrigo A. Cunha[3], Eugénia Carvalho[3,4], Carlos A. Fontes Ribeiro,[1] Flávio Reis[1] and Frederico C. Pereira [1,*]

1 Laboratory of Pharmacology and Experimental Therapeutics/IBILI, Faculty of Medicine, University of Coimbra, Subunit1-Pólo 3, Azinhaga de Santa Comba, Celas, 3000-354, Coimbra, Portugal;

2 Departamento de Farmacologia, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, UFSC, Florianópolis-SC 88049-900, Brazil;

3 CNC - Center for Neurosciences and Cell Biology, University of Coimbra, Largo Marquês de Pombal 3004-517 Coimbra, Portugal;

4 APDP, The Portuguese Diabetes Association, Rua do Salitre, nº. 118-120, 1250-203 Lisboa, Portugal

***Corresponding author full address:**

Frederico C. Pereira, PhD

Laboratory of Pharmacology and Experimental Therapeutics, IBILI,

Faculty of Medicine, Subunit 1 - Pólo 3,

3000-548 Coimbra, Portugal

Tel: +351 239 480071; Fax: +351 239 480065

E-mail:fredcp@ci.uc.pt

Abstract

Diabetes is associated with an increased risk for brain disorders, namely cognitive impairments associated with hippocampal dysfunction underlying diabetic encephalopathy. However, the impact of a prediabetes state on cognitive function is unknown. Therefore, we now investigated whether spatial learning and memory deficits and the underlying hippocampal dysfunction were already present in a prediabetes animal model. Adult Wistar rats drinking high-sucrose (HSu) diet (35% sucrose solution during nine weeks) were compared to controls drinking water. HSu rats exhibited fasting normoglycemia accompanied by hyperinsulinemia and hypertriglyceridemia in the fed state, and insulin resistance with impaired glucose tolerance confirming them as a prediabetes rodent model. HSu rats displayed a poorer performance in hippocampal-dependent short- and long-term spatial memory performance, assessed with the modified Y-maze and Morris water maze tasks, respectively; this was accompanied by a reduction of insulin receptor-β density with normal levels of insulin receptor substrate-1 pSer636/639, and decreased hippocampal glucocorticoid receptor levels without changes of the plasma corticosterone levels. Importantly, HSu animals exhibited increased hippocampal levels of AMPA and NMDA receptor subunits GluA1 and GLUN1, respectively, whereas the levels of proteins markers related to nerve terminals (synaptophysin) and oxidative stress/inflammation (HNE, RAGE, TNF-α) remained unaltered. These findings indicate that 9 weeks of sucrose consumption resulted in a metabolic condition suggestive of a prediabetic state, which translated into short- and long-term spatial memory deficits accompanied by alterations in hippocampal glutamatergic neurotransmission and abnormal glucocorticoid signaling.

Keywords

Diabetic encephalopathy, High-sucrose diet, Prediabetes, Hippocampus, Memory

Abbreviations: AUC, area under the curve; BBB, blood-brain barrier; Cont, control; GFAP, glialfibrillary acidic protein; GR, glucocorticoid receptor; GS, glutamine synthetase; GTT, glucose tolerance test; HbA1c, glycated hemoglobin; HOMA, homeostasis model assessment index; ITT, insulin tolerance test; HSu, high-sucrose; HNE, hydroxynonenal; LTP, long-term potentiation; T2DM, type 2 diabetes mellitus; RAGE, receptor for advanced glycation end products, TC, serum total cholesterol; TGs, triglycerides; TNF-α, tumor necrosis factor α.

1. Introduction

The development of type 2 diabetes mellitus (T2DM) is associated with an increased risk for brain disorders (Bruehl et al. 2009; Gold et al. 2007). In particular, a growing body of evidence indicates an increased risk of developing cognitive decline and dementia in a T2DM setting (Ravona-Springer et al. 2012; Roriz-Filho et al. 2009; Xu et al. 2010). T2DM triggers a condition of "diabetic encephalopathy" characterized by electrophysiological, structural and neurochemical changes leading to cognitive impairments (Biessels et al. 2002; Hernández-Fonseca et al. 2009; Mijnhout et al. 2006; Ristow 2004; Sima 2010). Indeed, memory deficits seem to be the most reliable altered cognitive function in T2DM and seem to have an early onset (Gold et al. 2007; Strachan et al. 1997; Winocur et al. 2005).

These T2DM cognitive deficits have been argued to be due in large part to an impaired central insulin modulation in the hippocampus, which is a critical region for memory processing (McNay and Recknagel 2011). In fact, adults with newly diagnosed prediabetes or T2DM show an insulin resistance associated with reductions in regional cerebral glucose metabolism and subtle cognitive impairments (Baker et al. 2011). Interestingly, the insulin signaling overlaps with pathways that regulate both synaptic plasticity and memory processes (Kamal et al. 2000; McNay and Recknagel 2011; van der Heide et al. 2006). Therefore, it is not surprising that insulin has effects on memory storage and synaptic physiology (Costello et al. 2012; McNay et al. 2010; van der Heide et al. 2006).

Accordingly, the preclinical animal studies investigating the relationship between T2DM and cognition have identified mild cognitive deficits (Bélanger et al. 2004; Duarte et al. 2012; Li et al. 2002; Winocur et al. 2005) typified by spatial learning and memory impairments in association with reduced hippocampal long-term potentiation, dendritic spine atrophy, decreased density of glutamatergic terminal markers and abnormal glutamatergic receptors regulation (Duarte et al. 2012; Trudeau et al. 2004). These diabetes-induced changes of

hippocampal-dependent memory and plasticity were proposed to result from the over-activation of the abundant hippocampal glucocorticoid receptors (GR) (Dorey et al. 2012; Sousa and Almeida, 2002) by the enhanced levels of corticosterone (Stranahan et al. 2008a) arising from an hyper-activation of the hypothalamic–pituitary–adrenal (HPA) axis that is characteristic of diabetes (Hwang et al. 2011; Stranahan et al. 2008a).

Diabetes is an evolving clinical situation, which is recognized to develop from a situation of metabolic impairment often named as prediabetic state (Tabák et al. 2012). The diagnostic criteria for prediabetes include one or more of the following: impaired fasting glucose [IFG, plasma glucose of 100 to 125 mg/dL (5.6 to 6.9 mmol/L)], impaired glucose tolerance [IGT, plasma glucose of 140 to 199 mg/dL (7.8 to 11.0 mmol/L) 2 hours after an oral load of 75 g dextrose] or hemoglobin A1c 5.7% to 6.4% (Tabák et al. 2012). Additionally, insulin resistance is already present in the pre-diabetic stage.

However, in contrast to T2DM, it is currently unknown if this condition of mild metabolic dysfunction is already associated with cognitive impairment. Therefore, the present study aimed at developing a model of metabolic dysfunction, based on the consumption of a high-sucrose (35% sucrose solution) diet during 9 weeks, to test if pre-diabetic rats displayed learning and memory deficits and an underlying hippocampal dysfunction. We found that metabolic changes suggestive of a pre-diabetic state translated into short- and long-term spatial memory deficits observed, respectively, in the Y-maze and Morris water maze tasks, and alterations on hippocampal glutamate receptors and GR levels.

2. Experimental Procedures

2.1. Animals and experimental procedures

Male Wistar rats (4 months-old) were obtained from Charles River Laboratories (Barcelona,

Spain). The animals were housed two per cage, under controlled environmental conditions [12 h light/dark cycle schedule under temperature (22±1 ºC) and humidity control]. After an adaptation period of 1 week, rats were randomly divided into two groups (n=8 animals per group), for a 9-weeks protocol: 1) control rats continued to drink tap water; 2) high-sucrose treated (HSu) rats received 35% sucrose (S0389; Sigma-Aldrich) in the drinking water. All animals were fed standard rat chow, containing 16.1% of protein, 3.1% of lipids, 3.9% of fibers and 5.1% of minerals (AO4 Panlab, Barcelona, Spain) *ad libitum* (with exception in the fasting periods). Food and beverage consumption was monitored for both groups throughout the experiment. The body weight of each animal was recorded weekly during the experimental period. All experiments were approved by the Institutional Animal Care and Use Committee from Faculty of Medicine, Coimbra University, and were performed following the European Community directive (2010/63/EU). All the animals were used for metabolic characterization (see Table 1) and behavioral assays, and within each group, 5 rats were used for neurochemical analysis.

2.2. Behavioral tasks

After 9 weeks, the short- and long-term spatial memories of control and HSu rats were assessed with a modified Y-maze and a Morris water maze, respectively. After habituation for at least 1 h before the beginning of the tests, behavior was monitored through a video camera positioned above the apparatuses and the images were later analyzed with the ANY Maze video tracking (Stoelting Co., Wood Dale, IL, USA) by an experienced investigator who was unaware of the experimental group being tested.

2.2.1. Water maze task

To evaluate the existence of long-term spatial memory deficits in HSu vs. control rats, the

animals were submitted to a spatial reference memory version of the water maze using a protocol described by Morris et al. (1982) and previously utilized in our laboratory (Castro et al. 2013). Tests were performed in a circular swimming pool made of black painted fibreglass, with 1.2 m internal diameter and 0.8 m height, and filled with water at 25°C to a depth of 0.6 m width. The target platform (10×10 cm) was made of transparent Plexiglas and was submerged 1–1.5 cm beneath the surface of the water. Starting points for the animals were marked on the outside of the pool as north (N), south (S), east (E) and west (W). Four distant visual cues (55×55 cm) were placed on the walls of the water maze room. They were all positioned with the lower edge 30 cm above the upper edge of the water tank, and in the standard setting the position of each symbol marked the midpoint of the perimeter of a quadrant (circle = NE quadrant, square = SE quadrant, cross = SW quadrant, and diamond = NW quadrant). The protocol consisted of 4 training days, four consecutive trials per day, during which the animals were left in the tank facing the wall, then being allowed to swim freely to the submerged platform placed in the centre of southwest quadrant of the tank. If the animal did not find the platform during a period of 60 s, it was gently guided to it. The animal was allowed to remain on the platform for 10 s after escaping to it and was then removed from the tank for 20 s before being placed at the next starting point in the tank. The apparatus was located in a room with indirect incandescent illumination. A monitor and a video-recording system were installed in an adjacent room. The experiments were video-taped and the scores for latency of escape from the starting point to the platform and swimming speed were later measured using the ANY-maze® video tracking system. The test session was carried out 24 h later and consisted of a single probe trial where the platform was removed from the pool and each rat was allowed to swim for 60 s in the maze. The time spent in the correct quadrant (i.e., where the platform was located on the training session) and in the inverse quadrant, the latency to platform zone and the number of crossings in the platform

zone were recorded.

2.2.2. Modified Y-maze task

The modified Y-maze was used to assess short-term spatial memory and it is based on the innate preference of animals to explore areas that have not been previously explored (Cognato et al. 2010). The Y-maze apparatus consisted of three arms (50 x 10 x 40 cm) made of wood covered with impermeable formica elevated to a height of 50 cm above the floor. This task consisted of two trials (training and test) of 5 min separated by an inter-trial interval of 90 min. During the training trial, one arm ("novel") was blocked by a removable door and the rat was placed into the end of the one arm ("start") facing the centre and it could chose between the start and the "other" arm. At the end of the training trial, the rat was removed from the maze and kept in an individual cage during the inter-trial interval (90 min). During the test trial, the "novel" arm was opened and the rat was once again placed in the start arm and allowed to explore the 3 arms during 5 min. The number of entries and the time spent in each arm were video monitored using ANY-maze TM tracking system. Entry into an arm was defined as placement of all 4 paws into the arm. The apparatus was cleaned with 10% ethanol between animals to avoid odor cues.

2.3. Blood and tissue collection and preparation

After the performance of the behavioral tasks, the animals were subjected to anesthesia with intraperitoneal (i.p.) injection of pentobarbital (50 mg/kg) (Sigma-Aldrich, Portugal) and blood samples were immediately collected by venipuncture from the jugular vein into syringes with Heparin-Lithium (Sarstedt, Monovette®) for plasma samples and into needles without anticoagulant for serum samples. Animals were then sacrificed by decapitation and the brains were immediately removed, placed in ice-cold Krebs buffer and carefully dissected.

Hippocampal regions were immediately frozen in liquid nitrogen and stored at -80 ºC until Western blot analyses.

2.4. Metabolic measurements

Glucose tolerance test (GTT) was performed in fasted rats (6-h) injected with glucose (2 g/kg, i.p.). The tail vein blood glucose levels were measured using a portable device (One Touch UltraEasy® glucometer, Lifescan, Johnson and Johnson, Portugal) in samples immediately before the bolus, 0 and 15, 30, 60, and 120 min after the bolus. Glycemia was also measured in fed conditions. Insulin tolerance test (ITT) was performed after a single injection of insulin (0.75 U/kg, i.p.) (I9278, Sigma), in 6-h fasted rats, through monitoring the blood glucose before, 0 and 15, 30, 45, 60 and 120 min following the insulin injection using the same glucometer. Fasting insulin levels were quantified by using a rat insulin ELISA kit (Mercodia, Uppsala, Sweden). Insulin sensitivity of individual animals was evaluated using the homeostasis model assessment (HOMA) index (Matthews et al. 1985). The formula used was as follows: [HOMA-IR] = fasting serum glucose (mg/dL) × fasting serum insulin (μU/mL) / 22.5. The values used (insulin and glucose) were obtained after an overnight fasting period.

Serum total cholesterol (TC) and triglycerides (TGs) were analyzed by enzymatic methods using an automatic analyzer (Hitachi 717, Roche Diagnostics). Total-cholesterol reagents and TGs kits were obtained from bioMérieux (Lyon, France). Corticosterone plasma levels were analyzed using an ELISA kit (ab108821, Abcam, Cambridge, UK).

2.5. Hippocampal neurochemical measurements

Total extracts were obtained from the left hippocampus as previously described (Simões et al. 2007). The hippocampus was homogenized in 400 μL of RIPA lysis buffer (150 mM NaCl; 50 mM Tris-HCl pH=8.0; 5 mM EGTA; 1% Triton X-100; 0.5% DOC; 0.1% SDS)

supplemented with a protease inhibitor cocktail (1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, 1 μg/mL chymostatin, 1 μg/mL leupeptin, 1 μg/mL antipain, 5 μg/mL pepstatin A, 50 mM sodium fluoride and 1 mM sodium orthovanadate (Sigma-Aldrich) and centrifuged (15000 *g*, 15 min, 4ºC), to discard insoluble material. Total protein concentration was determined using the bicinchonic acid protein assay kit (Thermoscientific®) (Smith et al. 1985) and supernatants were stored at -80 ºC until further use. Equal amounts of protein (5-75 μg) were loaded and separated by electrophoresis on sodium dodecyl sulfate polyacrylamide gel electrophoresis (7.5 - 12%), transferred to a 0.45 μm polyvinylidene difluoride (PVDF) membranes (Immobilon, Millipore, Madrid, Spain) and blocked with 1% bovine serum albumin (BSA) in phosphate buffer saline with 0.1% Tween-20 (PBS-T) for 1 h at room temperature. Membranes were then incubated overnight at 4ºC with the following primary antibodies against phospho-IRS-1 (Ser636/639, 1:1000), PSD-95 (1:1000) (both from Cell Signaling, MA, USA), IRS-1 (1:1000), GFAP (1:5000), GS (1:500), GluA1 (1:1000), GLUN1 (1:1000) and synpatophysin (1:1000) (all from Millipore MA, USA), RAGE (1:1000), TNF-α (1:600) (from Abcam, Cambridge, UK), IR-β (1:1000), GR (1:250) (from Santa Cruz, CA, USA) and HNE (1:1000) (from Calbiochem, Darmstadt, Germany). The membranes were washed extensively in 0.1% PBS-T and then incubated for 1 h at room temperature with alkaline phosphatase conjugated secondary antibodies [anti-rabbit and anti-mouse (1:5000) from GE Healthcare, Carnaxide, Portugal]. Finally, membranes were visualized using a Typhoon FLA 900 (GE Healthcare Bio-sciences) imaging system, using an enhanced chemifluorescence detection reagent (ECF, GE Healthcare). To confirm equal protein loading and sample transfer, membranes were re-probed with β-actin (1:10,000, from Sigma-Aldrich) or GAPDH (1:5000, from Abcam) antibodies. Densitometric analyses were performed using the Image Quant 5.0 software. Results were normalized against β-actin or GAPDH, and then expressed as percentage of control.

2.6. Statistical analysis

All values are expressed as means ± S.E.M. (*n* equals the number of rats). The comparisons of peripheral biochemical and hippocampal neurochemical changes and data of the Y-maze and probe test of the water maze were performed using an unpaired Student's *t*-test. The statistical analysis of the data of the metabolic (GTT, ITT) and water maze training was carried out using one- or two-way analysis of variance (ANOVA) followed by post-hoc Newman-Keuls multiple comparison test. Further statistical analyses were performed by one-sample *t*-test comparing for each group the % of time and % of entries in each arm during the training and test trials of the modified Y-maze and the % of time spent in the correct and the opposite quadrants during the probe test session of the Morris water maze with the respective chance level. The accepted level of significance for the tests was $P \leq 0.05$. All tests were performed using the GraphPad Prism 5.0 software for Windows.

3. Results

3.1. Characterization of the prediabetic state triggered by high-sucrose diet in rats

High-sucrose consumption (HSu) during 9 weeks did not influence the body weight of treated rats (Table 1). It is noteworthy that although HSu rats consumed more liquid ($P < 0.001$) compared to the control group (normal water), they ingested less chow ($P < 0.001$) (Table 1). However, HSu consumption induced an elevation on postprandial glycemia ($P < 0.05$) while leaving fasting glycemia unaltered compared to the control group (Table 1). Insulin levels were also influenced by sucrose consumption as demonstrated by a significant increase ($P < 0.001$) in serum insulin in the HSu group (Table 1). When focusing on glucose tolerance, AUC-GTT from HSu rats was significantly higher ($P < 0.05$) compared to the control group (Table 1). Corroborating these observations, 120 min after insulin

administration, HSu rats displayed significantly higher blood glucose levels in the ITT ($P < 0.001$) and HOMA-IR ($P < 0.01$) than control rats, thus confirming the insulin resistance (Table 1). Furthermore, glycated hemoglobin (HbA1c) from HSu rats was higher ($P < 0.05$) compared to controls (Table 1). HSu rats also showed elevated plasma triglycerides ($P < 0.05$) but normal plasma total cholesterol ($P > 0.05$) levels when compared to the control group (Table 1). Additionally, plasma corticosterone levels were also normal (Table 1).

3.2.The prediabetes state disrupted short- and long-term spatial memory

Two-way ANOVA (treatment *vs*. repeated measures) revealed no significant differences [$F(1, 13)=0.25$, $P=0.62$] between control and HSu rats to acquire the spatial information in the water maze, as indicated by similar escape latencies to find the platform during the training sessions (Fig. 1A). Moreover, one-sample *t*-test indicated that control rats spent more time in the correct quadrant (Fig. 1B) and less time in the opposite quadrant (Fig. 1C) in comparison to chance performance (25%) during the probe test of the water maze (without platform), indicating that control rats were able to remember the platform location during the probe test session in the next day. On the other hand, a Student's *t*-test indicated that during the probe test, HSu rats spent less time in the correct quadrant (Fig. 1B), more time in the opposite quadrant (Fig. 1C), had a higher latency to reach the platform zone (Fig. 1D) and a reduced number of crossings in the platform zone (Fig. 1E) when compared to control rats. Altogether, these results indicate a selective deficit of long-term spatial memory, but not in spatial learning, after 9 weeks of high sucrose diet in rats.

Since the performance in the modified Y-maze task is dependent of the exploratory behavior of the animals, we first evaluated the locomotor activity in the training trial. No significant differences were observed between control and HSu rats in the number of entries and the time spent in the two arms ("start" and "other") ($P > 0.05$; Fig. 2A, B, C). One-sample

t-test analysis revealed that only control rats were able to recognize the "novel" arm as the unvisited arm in the previous trial, as indicated by the significant increase in the percentage of entries (Fig. 2E) and time (Fig. 2F) in the "novel" arm in comparison to chance performance (33.3%). More importantly, a Student's *t*-test revealed short-term spatial memory deficits in HSu-treated rats as indicated by a significant reduction of the percentage of entries and time in the "novel" arm in comparison to control group ($P < 0.05$**;** Fig. 2E, F).

3.3. Characterization of hippocampal alterations triggered by the high-sucrose diet

3.3.1. The pre-diabetes state decreased the density of insulin receptors (IR-β) in the hippocampus

The suggested link between cognitive deficits and central insulin signaling led us to evaluate insulin markers in the hippocampus. High-sucrose consumption during 9 weeks induced a significant decrease in hippocampal IR-β levels (80.0±5.1%, n=5; $P < 0.05$) compared to controls (Fig. 3A). However, the immunoreactivity of both IRS-1 and IRS-1-pSer636/639 in the hippocampus was not significantly altered ($P > 0.05$) in the HSu group (Fig. 3B,C).

3.3.2. The pre-diabetes state induced an increase in the density of GluA1 and GLUN1 in the hippocampus

The spatial memory deficits observed in HSu rats warranted the study of hippocampal GluA1 and GLUN1 levels on account of their critical role in synaptic plasticity. The hippocampal levels of both GluA1 and GLUN1 were significantly increased in HSu rats (130.5±2.8%, n=5 and 152.1±5.3%, n=5, respectively; $P < 0.05$) when compared to control rats (Fig. 4 A,B). On the other hand, the levels of the post-synaptic glutamatergic marker

(PSD-95) remained unaltered ($P > 0.05$; Figure 4C). Moreover, the immunoreactivity of synaptophysin (a pre-synaptic vesicle protein) remained unchanged in the hippocampus from HSu compared to control rats ($P > 0.05$; Figure 4D).

3.3.3. The pre-diabetes state induced a decrease in the density of GR in the hippocampus

Since abnormal corticosterone signaling in the hippocampus has been proposed to underlie diabetes-associated memory impairment, we probed the density of glucocorticoid receptors (GR) in the hippocampus of HSu rats. It was found that the levels of GR were significantly decreased in the hippocampus of HSu rats when compared to control rats (60.0 ± 5.2%, $P < 0.05$; Figure 4E).

3.3.4. Evaluation of hippocampal oxidative stress/inflammation markers upon high-sucrose diet

Both oxidative stress and inflammation are two key players in diabetic encephalopathy. Therefore it was important to assess the levels of hydroxynonenal (HNE, a precursor for advanced lipoxidation end product-ALE), RAGE (receptor for advanced glycation end products), TNF-α, GFAP and GS (astrogliosis markers) in the hippocampus of HSu rats. However, no significant differences in any of these parameters were observed after 9 weeks of high-sucrose exposure compared to control rats (Table 2).

4. Discussion

Our results show for the first time that a pre-diabetes state in rats triggers short- and long-term spatial memory deficits observed in the modified Y-maze and Morris water maze

tasks, respectively, that are accompanied by a decrease of the levels of insulin receptors, changes in glutamatergic neurotransmission and a decrease of the levels of glucocorticoid receptor in the hippocampus.

Diet is an important environmental determinant for life-style-related diseases such as T2DM (Steyn et al. 2004). Although numerous studies with rodents have already shown that a high-sucrose diet (HSu) induces insulin resistance and hypertriglyceridemia (Carvalho et al. 2012; Conde et al. 2011; Kanazawa et al. 2003; Ribeiro et al. 2005; Sumiyoshi et al. 2011), the impact on brain functioning of pre-diabetic animal models, namely the HSu-treated rat, are scarce. The current findings indicate no significant differences on glycemia in the fasting state, but a marked increase in the fed glycemia (postprandial) in HSu-treated rats, together with impaired glucose tolerance (IGT), hyperinsulinemia and insulin resistance, all characteristic of a metabolic disease-like pre-diabetic condition (Tabák et al. 2012). These metabolic changes are in agreement with previous studies demonstrating sucrose-induced insulin resistance in rats with fasting normoglycemia (Thresher et al. 2000). Additionally, this pre-diabetic model is characterized by hypertriglyceridemia, without obesity and hypertension, as previously documented by other authors (Cao et al. 2007; Carvalho et al. 2012; Kanazawa et al. 2003; Ribeiro et al. 2005; Santuré et al. 2002; Sumiyoshi et al. 2011). Taken together, these results confirm the current approach of high sucrose consumption during 9 weeks as a valuable model of pre-diabetes/insulin resistance, characterized by fasting normoglycemia, IGT, hyperinsulinemia, insulin resistance and hypertriglyceridemia, without obesity and hypertension.

Some studies have linked insulin resistance and T2DM to deficits of hippocampal-dependent memory function (Convit, 2005; Gold et al. 2007; Strachan et al. 1997; Winocur et al. 2005). Importantly, Gold et al. (2007) highlighted that memory impairments associated with hippocampal alterations represent some of the early brain complications in T2DM. In

accordance with this view, our results showed that a pre-diabetic state disrupted hippocampal-dependent short- and long-term spatial memories in rats evaluated in the modified Y-maze and Morris water maze tasks, respectively, without major alterations on spatial learning and locomotor activity. It is noteworthy that the modified Y-maze and the spatial version of the Morris water maze tests reliably probe, respectively, short- and long-term spatial memory performance (Castro et al. 2013; Duarte et al. 2006, 2012; Prediger et al. 2006). In fact, in both tests, the animals need to make associations among the spatial environmental cues to form a cognitive map that helps them to find the platform localization (Morris et al. 1982) or the previously unvisited arm (Dellu et al. 1997). Importantly, this modified Y maze test is non-aversive since it does not require either food deprivation (as opposed to the radial maze) or electrical foot-shock (as opposed to inhibitory avoidance task), which could modify the motivational and emotional status of the animal (Bekker et al. 2006), thus confounding the spatial memory parameters measured.

Memory deficits seen herein corroborate previous observation by Chepulis et al. (2009) that 9 and 12 months of exposure to 7.9% sucrose induced a significant decrease in the proportion of rats that recognized the novel arm as the unvisited arm of the Y-maze when compared to controls. Interestingly, Cao et al. (2007) reported insulin resistance and exacerbation of memory deficits in a transgenic mouse model of Alzheimer following 25 weeks of 10%-sucrose-sweetened water intake. Moreover, other types of diet, including high-fructose, that induce metabolic alterations were also able to promote marked memory impairments (McNay et al. 2010; Mielke et al. 2005; Molteni et al. 2002; Ross et al. 2009; Stranahan et al. 2008b; Winocur and Greenwood, 1999; Wu et al. 2003). Other studies showed that high-fat diets (41-59%; HFD) inducing stronger metabolic alterations compared with our model were not always associated with cognitive impairments (Leboucher et al. 2013; Pistell et al. 2010). However, it should be stressed that these studies used a different

species (mice), behavioral paradigms [mean acquisition errors/Stone T-maze (Pistell et al. 2010) and escape latency/path length /Morris water maze (Leboucher et al. 2013) as spatial learning read-outs] and different ages. In fact, Pistell and collaborators (2010) acknowledged that cognitive performance is known to decrease with age and that 12 month-old "control" mice might have mild impairments compared to younger mice thus masking a putative effect of a 41% fat regimen. They further indicated the need for a systematic evaluation of how increasing age might modulate cognitive function. Moreover, in contrast to our study, both studies only assessed spatial learning whereas we evaluated both spatial learning and memory. Notably, in our study, HSu also failed to display learning impairment as gauged by similar escape latency to find the platform during the training trials of the Morris water maze when compared with controls. Therefore one cannot exclude that these HFD could also induce spatial memory deficits as demonstrated by Kosary et al. (2012) using both Y maze and novel object recognition tests.

It is worth noting that the present study cannot rule out the hypothesis that the fructose moiety of the sucrose disaccharide crosses the blood-brain barrier (BBB) (Cha and Lane, 2009) and may be accountable for sucrose-induced memory impairments observed in the current study. Furthermore, hypertriglyceridemia seen in the HSu group that could have been triggered by fructose may also contribute to the memory dysfunction observed herein. In support of this idea, direct injection of triglycerides into the brain has detrimental consequences for learning and memory (Farr et al. 2008) and insulin transport through the BBB (Banks et al. 2004; Banks et al. 2008; Urayama et al. 2008).

Overall, it has been suggested that these memory deficits are probably due to a disruption of insulin signaling (Ristow, 2004; van der Heide et al. 2006). Considering that chronic peripheral hyperinsulinemia may down-regulate BBB insulin receptors thus reducing insulin transport into the brain (Banks 2004; Craft and Watson 2004; Wallum et al. 1987), we

cannot rule out the possibility of a reduction of hippocampal insulin levels in HSu animals. However, hippocampal insulin signaling can also be sustained by insulin synthesized by hippocampal pyramidal neurons (Kuwabara et al. 2011). There is no previous study addressing the impact of high sucrose treatment on the ability of hippocampal cells to produce insulin. Besides insulin levels, insulin signaling could also be affected in the hippocampus thus contributing to cognitive deficits exhibited by HSu rats. Indeed, we found a reduction in IR-β levels in HSu compared to control rats. This is consistent with IR expression being diminished in type-2 diabetic patients (Nisticò et al. 2012). On the other hand, Winocur and colleagues (2005) found no differences in the hippocampal IR-β expression on hyperinsulinemic ZDF rats, with 6 months of age. Moreover this decrease of IR-β levels in HSu rats was accompanied by a lack of alteration of IRS-1 pS636/639 levels, which is a known negative regulator of IRS-1 (Talbot et al. 2012). Therefore, our results suggest that, although there was a downregulation of the insulin receptor, hippocampal insulin response might not be affected. However, we cannot exclude that IRS-2 signaling could be compromised in HSu animals, since it was recently demonstrated that this insulin receptor substrate is also involved in hippocampal synaptic plasticity (Costello et al. 2012).

Impaired cognitive performance is also associated with disglycemia (Gao et al. 2008). One of the major key players that translate hyperglycemia into glucotoxicity is RAGE-mediated inflammatory/oxidative stress pathways (Ramasay et al. 2005). Additionally, RAGE–mediated pathways have been implicated in memory deficits in Alzheimer's disease (Arancio et al. 2004; Fang et al. 2010; Maczurek et al. 2008; Wilson, 2009). Nevertheless, sucrose-sweetened water did not increase fasting glycemia and, thus, glucose neurotoxicity ought not be held responsible for the memory deficits reported here. The RAGE levels in HSu rats were comparable to control rats, which is consistent with normoglycemia. Furthermore, we failed to observe any sign of hippocampal oxidative stress in such early pre-diabetic stage,

as gauged from the normal HNE found in the hippocampi of HSu rats when compared with control rats. The key pro-inflammatory cytokine TNF-α was found to be over-expressed in diabetic hippocampi and was coupled with cognitive dysfunction (Liu et al. 2012). Additionally, RAGE up-regulation increases TNF-α levels (Ramasay et al. 2005). Consistently with RAGE data, TNF-α levels from HSu rats were not significantly different from control animals. The absence of an oxidative stress profile along with normal TNF-α levels is consistent with the lack of synaptotoxicity as seen by normal synaptophysin levels in HSu when compared to control rats. Upon neurotoxicity, astrocytes can become reactive (Pekny and Wilson, 2005). Therefore, it is not surprising that we did not observe significant difference in the hippocampal GFAP and GS levels between HSu and control groups. Contrasting with our findings, Duarte et al., (2012) recently showed the association between the synaptotoxicity and astrogliosis with T2DM-induced memory impairment. However one should stress that these authors used a diabetic phenotype characterized by hyperglycemia whereas our model is normoglycemic, which suggests that astrogliosis is a feature characteristic of T2DM rather than of pre-diabetes.

Other mechanisms underlying memory deficits should be considered. For example, it is now well established that adaptive changes in glutamatergic synapses, typified by modified densities of NMDAR and AMPAR, are tightly associated with synaptic plasticity and memory (Yashiro and Philpot, 2008; Santos et al. 2009). Although memory impairments and hippocampal glutamargic dysfunction have been associated with diabetic encephalopathy characteristic of T2DM (Trudeau et al. 2004), nothing is known about the regulation of GluA1 and GLUN1 subunits, that are crucial for synaptic plasticity phenomena (Lee, 2006), in pre-diabetic conditions. Remarkably, we now found an up-regulation of GluA1 and GLUN1 subunits in hippocampal total extracts of HSu compared to control rats. However, these alterations were not accompanied by significant changes on PSD-95, one of the

fundamental glutamatergic scaffolding proteins. This suggests that HSu rats have normal glutamatergic synaptic density. The up-regulation of both AMPA/NMDA subunits might be a compensatory mechanism to circumvent their defective activity underlying memory impairment. Interestingly, Turringiano and colleagues showed that the inhibition of synaptic transmission up-regulates AMPAR transcription (Turrigiano et al., 1998), presumably as a means of compensation. It is noteworthy that an up-regulation of both NMDA and AMPA receptors in thoracic spinal cord sections was observed at early stages of a mouse model that closely resembles type 2 (insulin-independent) diabetes of obese-diabetic ob/ob mice (Li et al., 1999). It was also reported that the up-regulation of hippocampal glutamate NMDAR and AMPAR accompanied synaptic plasticity defects in T1DM animal models as reviewed by Trudeau et al. (2004). Moreover memory impairments observed in this pre-diabetes state may also be underlined by changes in other ionotropic glutamate receptors (eg. GluA2, GluN2A and GluN2B) levels as well as in their phosphorylation status and/or subcellular localization as observed in T1DM and T2DM experimental models (Di Luca et al. 1999; Trudeau et al. 2004).

Aberrant corticosterone signaling was also implied in diabetes induced memory deficits. In fact, Strahan et al. (2008a) demonstrated that high levels of circulating corticosterone contributed to diabetes impaired hippocampal-dependent memory and synaptic plasticity in both insulin–deficient rats and insulin–resistant (ob/ob) mice. Moreover it was recently shown that a GR antagonist (mifepristone) ameliorated cognitive dysfunction in streptozotocin (STZ)-induced type-1 diabetic rats (Zuo et al. 2011). However there is conflicting data on GR levels in diabetic models. While Goto–Kakizaki rats exhibited decreased GR immunolabeling in the CA1 area, associated with higher corticosteronemia (Beauquis et al. 2010), ZDF rats exhibited an increase in hippocampal GR levels (Hwang et al. 2011). Moreover, Shin et al. (2013) showed recently that hippocampal GR protein expression increased significantly until

the 3rd week, but decreased at the 4th week following STZ administration. At this moment, there is no information regarding corticosterone circulating levels and hippocampal GR levels in a pre-diabetic state. Notably, we now show that consumption of high sucrose for 9 weeks significantly decreased GR hippocampal levels when compared to control rats. Interestingly, this GR down-regulation was accompanied by normal corticosterone plasmatic levels. Whereas it is not unexpected that glucorticoids are still normal in a pre-diabetes state, this decrement of GR levels might lead to failure of feedback regulation of the HPA axis thereby contributing to mounting glucocorticoid levels putatively seen when evolving to a diabetes state. This mechanism was proposed to be operative in an early phase of Alzheimer's disease mouse model where GR down-regulation coincided with the onset of memory decline in the object recognition test and preceded the increase in plasma levels of corticosterone (Escribano et al. 2008). Therefore, one cannot exclude that the adaptative change of hippocampal GR density seen herein might have contributed to the glutamatergic dysfunction and/or memory impairment exhibited by HSu rats.

In conclusion, in the present study we confirm the deleterious effect of adding sucrose to a normal rodent diet, resulting in a pre-diabetic state, mainly characterized by fasting normoglycemia, hyperinsulinemia, insulin resistance, hypertriglyceridemia and impaired glucose tolerance compared with the control rats. Notably, we provide evidence showing that this condition of pre-diabetes was already associated with short- and long-term spatial memory impairments, which were underlined by a compromised glutamatergic as well as glucocorticoid function in the hippocampi from HSu rats. These data reinforce the suggestion that cognitive impairment is an early feature of T2DM, since it is already observed in conditions mimicking metabolic disease. Furthermore, it highlights the potential role of dietary sugar in the early central diabetic complications and suggests that controlling the consumption of sugar-sweetened beverages may be an effective way to curtail the risk of

developing T2DM. In this context, the identification of the mechanisms by which glutamatergic as well as glucocorticoid signaling contribute to "diabetic encephalopathy" might be of paramount clinical relevance.

Conflict of Interest Statement

None.

Authors´ contribution

ES, FR and FCP designed the study protocol. ES, RDP and AAC performed the behavioral experiments. FCP, FR, ES, CL, CMDS, PMA, SN and SDV collected blood and brain samples and performed metabolic and hippocampal measurements. ES, RDP, EC, FR and FCP analysed data. ES, RDP and FCP wrote the paper. EC, RAC, CFR and FR contributed with scientific expertise and revisions of the paper. All authors have read and approved the manuscript.

Acknowledgments

This research was supported by PEst-C/SAU/UI3282/2011 (Faculty of Medicine, University of Coimbra, Portugal) and PTDC/SAU-OSM/104124/2008 (E.C). SDV is a recipient of a PhD grant from Fundação para a Ciência e a Tecnologia (FCT, Portugal, SFRH/BD/78166/2011). We would also like to thank Dr. Ana Luisa Carvalho (CNC - Center for Neurosciences and Cell Biology, University of Coimbra) and Dr. Francisco Ambrósio (IBILI, Faculty of Medicine, University of Coimbra) for kindly offering GluA1, GluN1, synaptophyisin and PSD-95 antibodies.

References

Arancio O, Zhang HP, Chen X, Lin C, Trinchese F, Puzzo D, Liu S, Hegde A, Yan SF, Stern A, Luddy JS, Lue LF, Walker DG, Roher A, Buttini M, Mucke L, Li W, Schmidt AM, Kindy M, Hyslop PA, Stern DM, Du Yan SS (2004) RAGE potentiates Abeta-induced perturbation of neuronal function in transgenic mice. EMBO J 23:4096–4105.

Banks WA, Coon AB, Robinson SM, Moinuddin A, Shultz JM, Nakaoke R, Morley JE (2004) Triglycerides induce leptin resistance at the blood-brain barrier. Diabetes. 53: 1253-1260.

Banks WA, Burney BO, Robinson SM (2008) Effects of triglycerides, obesity, and starvation on ghrelin transport across the blood-brain barrier. Peptides. 29: 2061-2065.

Baker LD, Cross D, Minoshima S, Belongia D, Stennis G, Watson, Craft S (2011) Insulin resistance and Alzheimer-like reductions in regional cerebral glucose metabolism for cognitively normal adults with prediabetes or early type 2 diabetes. Arch Neurol 68: 51–57.

Bekker A, Shah R, Quartermain D, Li YS, Blanck T (2006) Isoflurane preserves spatial working memory in adult mice after moderate hypoxia. Anesth Analg 102: 1134-1138.

Bélanger A, Lavoie N, Trudeau F, Massicotte G, Gagnon S (2004) Preserved LTP and water maze learning in hyperglycaemic–hyperinsulinemic ZDF rats. Physiol Behav 83: 483–494.

Beauquis J, Homo-Delarche F, Giroix M-H, Ehses J, Coulaud J, Roig P, Portha B, De Nicola AF, Saravia F (2010) Hippocampal neurovascular and hypothalamic–pituitary–adrenal axis alterations in spontaneously type 2 diabetic GK rats. Exp Neurol 222: 125–134.

Biessels GJ, van der Heide L, Kamal A, Bleys R, Gispen W (2002) Ageing and diabetes: implications for brain function. Eur J Pharmacol 44: 1-14.

Bruehl H, Wolf OT, Sweat V, Tirsi A, Richardson S, Convit A (2009) Modifiers of cognitive function and brain structure in middle-aged and elderly individuals with type 2 diabetes mellitus. Brain Research 1280: 186–194.

Cao D, Lu H, Lewis TL, Li L (2007) Intake of sucrose-sweetened water induces insulin resistance and exacerbates memory deficits and amyloidosis in a transgenic mouse model of Alzheimer disease. J Biol Chem 282: 36275-36282.

Carvalho C, Cardoso S, Correia SC, Santos RX, Santos MS, Baldeiras I, Oliveira CR, Moreira PI (2012) Metabolic alterations induced by sucrose intake and Alzheimer's

disease promote similar brain mitochondrial abnormalities. Diabetes 61: 1234-1242.

Castro AA, Wiemes BP, Matheus FC, Lapa FR, Viola GG, Santos AR, Tasca CI, Prediger RD (2013) Atorvastatin improves cognitive, emotional and motor impairments induced by intranasal 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration in rats, an experimental model of Parkinson's disease. Brain Res 1513: 103-116.

Cha SH, Lane MD (2009) Central lactate metabolism suppresses food intake via the hypothalamic AMP kinase/malonyl-CoA signaling pathway. Biochem Biophys Res Commun 386: 212-216.

Chepulis LM, Starkey NJ, Waas JR, Molan PC (2009) The effects of long-term honey, sucrose or sugar-free diets on memory and anxiety in rats. Physiol Behav 97: 359-368.

Cognato GP, Agostinho PM, Hockemeyer J, Müller CE, Souza DO, Cunha RA (2010) Caffeine and adenosine A_{2A} receptor antagonists prevent memory impairment and synaptotoxicity in adult rats triggered by a convulsive episode in early life. J Neurochem 112: 453-462.

Conde SV, Nunes da Silva T, Gonzalez C, Mota Carmo M, Monteiro EC, Guarino MP (2011) Chronic caffeine intake decreases circulating catecholamines and prevents diet-induced insulin resistance and hypertension in rats. Br J Nutr 1071: 86-95.

Convit A (2005) Links between cognitive impairment in insulin resistance: an explanatory model. Neurobiol Aging 26: 31–35.

Costello DA, Claret M, Al-Qassab H, Plattner F, Irvine EE, Choudhury AI, Giese P, Withers DJ, Pedarzani P (2012) Brain deletion of insulin receptor substrate 2 disrupts hippocampal synaptic plasticity and metaplasticity. PLoS One 7: 31124.

Craft S, Watson GS (2004) Insulin and neurodegenerative disease: shared and specific mechanisms. Lancet Neurol 3: 169-178.

Cumiskey D, Butler MP, Moynagh PN, O'Connor JJ (2007) Evidence for a role for the group I metabotropic glutamate receptor in the inhibitory effect of tumor necrosis factor-alpha on long-term potentiation. Brain Res 1136: 13–19.

Dellu F, Fauchey V, LeMoal M, Simon H (1997) Extension of a new two-trial memory task in the rat: influence of environmental context on recognition processes. Neurobiol Learn Mem 67: 112–120.

Di Luca M, Ruts L, Gardoni F, Cattabeni F, Biessels GJ, Gispen WH (1999) NMDA receptor subunits are modified transcriptionally and post-translationally in the brain of streptozotocin-diabetic rats. Diabetologia 42: 693-701.

Dorey R, Piérard C, Chauveau F, David V, Béracochéa D (2012) Stress-induced memory retrieval impairments: different time-course involvement of corticosterone and glucocorticoid receptors in dorsal and ventral hippocampus. Neuropsychopharmacology 37: 2870–2880.

Duarte JM, Oliveira CR, Ambrósio AF, Cunha RA (2006) Modification of adenosine A_1 and A_{2A} receptor density in the hippocampus of streptozotocin-induced diabetic rats. Neurochem Int 48: 144–150.

Duarte JM, Carvalho RA, Cunha RA, Gruetter R (2009) Caffeine consumption attenuates neurochemical modifications in the hippocampus of streptozotocin-induced diabetic rats. J Neurochem 111: 368–379.

Duarte JM, Agostinho PM, Carvalho RA, Cunha RA (2012) Caffeine consumption prevents diabetes-induced memory impairment and synaptotoxicity in the hippocampus of NONcZNO10/LTJ mice. PLoS One 7: 21899.

Escribano L, Simón A-M, Pérez-Mediavilla A, Salazar-Colocho P, Del Río J, Frechilla D (2009) Rosiglitazone reverses memory decline and hippocampal glucocorticoid receptor down-regulation in an Alzheimer's disease mouse model. Biochem Biophys Res Commun 379: 406–410.

Fang F, Lue LF, Yan S, Xu H, Luddy JS, Chen D, Walker DG, Stern DM, Schmidt AM, Chen JX, Yan SS (2010) RAGE-dependent signaling in microglia contributes to neuroinflammation, Abeta accumulation, and impaired learning/memory in a mouse model of Alzheimer's disease. FASEB J 24: 1043–1055.

Farr SA, Yamada KA, Butterfield DA, Abdul HM, Xu L, Miller NE, Banks WA, Morley JE (2008) Obesity and hypertriglyceridemia produce cognitive impairment. Endocrinology 149: 2628-2636.

Gao L, Matthews FE, Sargeant LA, Brayne C (2008) An investigation of the population impact of variation in HbA1c levels in older people in England and Wales: From a population based multi-centre longitudinal study. BMC Public Health 8: 54.

Gold S, Dziobek I, Sweat V, Tirsi A, Rogers K, Bruehl H, Tsui W, Richardson S, Javier E, Convit A (2007) Hippocampal damage and memory impairments as possible early brain complications of Type 2 Diabetes. Diabetologia 50: 711–719.

Hernández-Fonseca J, Rincón J, Pedreañez A, Viera N, Arcaya J, Carrizo E, Mosquera J (2009) Structural and ultrastructural analysis of cerebral cortex, cerebellum, and hypothalamus from diabetic rats. Exp Diabetes Res 329632: 1-12.

Hwang IK, Yi SS, Yoo K-Y, Park OK, Yan B, Song W, Won M-H, Yoon YS, Seong JK (2011) Effect of treadmill exercise on blood glucose, serum corticosterone levels and glucocorticoid receptor immunoreactivity in the hippocampus in chronic diabetic rats. Neurochem Res 36: 281–287.

Kamal A, Biessels GJ, Duis SE, Gispen WH (2000) Learning and hippocampal synaptic plasticity in streptozotocin-diabetic rats: interaction of diabetes and ageing. Diabetologia 43: 500-506.

Kanazawa M, Xue CY, Kageyama H, Suzuki E, Ito R, Namba Y, Osaka T, Kimura S, Inoue S (2003) Effects of a high-sucrose diet on body weight, plasma triglycerides, and stress tolerance. Nutr Rev 61: S27-S33.

Kosari S, Badoer E, Nguyen JCD, Killcross AS, Jenkins TA (2012) Effect of western and high fat diets on memory and cholinergic measures in the rat. Behav Brain Res 235: 98-103.

Kuwabara T, Kagalwala MN, Onuma Y, Ito Y, Warashina M, Terashima K, Nakashima K, Gage FH, Asashima M (2011) Insulin biosynthesis in neuronal progenitors derived from adult hippocampus and the olfactory bulb. EMBO Mol Med 3: 742–754.

Lee HK (2006) AMPA receptor phosphorylation in synaptic plasticity: insights from knockin mice, in: Kittler J.T., Moss S.J. (Eds), The dynamic synapse: molecular methods in ionotropic receptor biology. Frontiers in Neuroscience, Boca Raton (FL): CRC Press.

Li N, Young MM, Bailey CJ, Smith ME (1999) NMDA and AMPA glutamate receptor subtypes in the thoracic spinal cord in lean and obese-diabetic ob/ob mice. Brain Res. 849: 34– 44.

Li B, Chen N, Luo T, Otsu Y, Murphy TH, Raymond LA (2002) Differential regulation of synaptic and extra-synaptic NMDA receptors. Nat Neurosci 5: 833-834.

Liu YW, Zhu X, Li W, Lu Q, Wang JY, Wei YQ, Yin XX (2012) Ginsenoside Re attenuates diabetes associated cognitive deficits in rats. Pharmacol Biochem Behav 101:93–98.

Maczurek A, Shanmugam K, Münch G (2008) Inflammation and the redox-sensitive AGE-RAGE pathway as a therapeutic target in Alzheimer's disease. Ann NY Acad Sci 1126: 147-151.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentration in man. Diabetologia 28: 412–419.

McNay EC, Ong CT, McCrimmon RJ, Cresswell J, Bogan JS, Sherwin RS (2010) Hippocampal memory processes are modulated by insulin and high-fat-induced insulin resistance. Neurobiol Learn Mem 93: 546-553.

McNay EC, Recknagel AK (2011) Brain insulin signaling: A key component of cognitive processes and a potential basis for cognitive impairment in type 2 diabetes. Neurobiol Learn Mem 96: 432-442.

Mielke JG, Taghibiglou C, Liu L, Zhang Y, Jia Z, Adeli K, Wang YT (2005) A biochemical and functional characterization of diet-induced brain insulin resistance. J Neurochem 93: 1568–1578.

Mijnhout GS, Scheltens P, Diamant M, Biessels GJ, Wessels AM, Simsek S, Snoek FJ, Heine RJ (2006) Diabetic encephalopathy: a concept in need of a definition. Diabetologia 49: 1447–1448.

Molteni R, Barnard RJ, Ying Z, Roberts CK, Gómez-Pinilla F (2002) A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. Neuroscience 112: 803-814.

Morris RG, Garrud P, Rawlins JN, O'Keefe J (1982) Place navigation impaired in rats with hippocampal lesions. Nature 297: 681-683.

Nisticò R, Cavallucci V, Piccinin S, Macrì S, Pignatelli M, Mehdawy B, Blandini F, Laviola G, Lauro D, Mercuri NB, D'Amelio M (2012) Receptor β-subunit haploinsufficiency impairs hippocampal late-phase LTP and recognition memory. Neuromolecular Med 14: 262-269.

Pekny M, Nilsson M (2005) Astrocyte activation and reactive gliosis. Glia 50: 427–434.

Prediger RD, Batista LC, Medeiros R, Pandolfo P, Florio JC, Takahashi RN (2006) The risk is in the air: Intranasal administration of MPTP to rats reproducing clinical features of Parkinson's disease. Exp Neurol 202: 391-403.

Ramasamy R, Vannucci SJ, Yan SS, Herold K, Yan SF, Schmidt AM (2005) Advanced glycation end products and RAGE: a common thread in aging, diabetes, neurodegeneration, and inflammation. Glycobiology 15: 16-28.

Ravona-Springer R, Moshier E, Schmeidler J, Godbold J, Akrivos J, Rapp M, Grossman HT, Wysocki, M, Silverman JM, Haroutunian V, Beeri MS (2012). Changes in glycemic control are associated with changes in cognition in non-diabetic elderly. J Alzheimers Dis 30: 299-309.

Ribeiro RT, Lautt WW, Legare DJ, Macedo MP (2005) Insulin resistance induced by sucrose feeding in rats is due to an impairment of the hepatic parasympathetic nerves. Diabetologia 48: 976-983.

Ristow M (2004) Neurodegenerative disorders associated with diabetes mellitus. J Mol Med 82: 510-529.

Roriz-Filho J, Sá-Roriz TM, Rosset I, Camozzato AL, Santos AC, Chaves ML, Moriguti JC, Roriz-Cruz M (2009) (Pre)diabetes, brain aging, and cognition. Biochim Biophys Acta 1792: 432-443.

Ross AP, Bartness TJ, Mielke JG, Parent MB (2009) A high fructose diet impairs spatial memory in male rats. Neurobiol Learn Mem 92: 410–416.

Santos SD, Carvalho AL, Caldeira MV, Duarte CB (2009) Regulation of AMPA receptors and synaptic plasticity. Neuroscience 158: 105-125.

Santuré M, Pitre M, Marette A, Deshaies Y, Lemieux C, Larivière R, Nadeau A, Bachelard H (2002) Induction of insulin resistance by high-sucrose feeding does not raise mean arterial blood pressure but impairs hemodynamic responses to insulin in rats. Br J Pharmacol 137: 185-196.

Shin JH, Seong JK, Yi SS (2013) Sequential alterations of glucocorticoid receptors in the hippocampus of STZ-induced type 1 diabetic rats. J Vet Sci (in press).

Sima AA (2010) Encephalopathies: the emerging diabetic complications. Acta Diabetol 47: 279-293.

Simões PF, Silva AP, Pereira FC, Marques E, Grade S, Milhazes N, Borges F, Ribeiro CF, Macedo TR. (2007) Methamphetamine induces alterations on hippocampal NMDA and AMPA receptor subunit levels and impairs spatial working memory. Neuroscience 150: 433-441.

Smith P, Krohn R, Hermanson G, Mallia A, Gartner F, Provenzano M, Fujimoto E, Goeke N, Olson B, Klenk D (1985) Measurement of protein using bicinchoninic acid. Anal Biochem 150: 76–85.

Sousa N, Almeida OF (2002) Corticosteroids: sculptors of the hippocampal formation. Rev Neurosci 13: 59–84.

Steyn NP, Mann J, Bennett PH, Temple N, Zimmet P, Tuomilehto J, Lindström J, Louheranta A (2004) Diet, nutrition and the prevention of type 2 diabetes. Public Health Nutr 7: 147-165.

Strachan M, Deary I, Ewing F, Frier B (1997) Is type II diabetes associated with an increased risk of cognitive dysfunction? A critical review of published studies. Diabetes Care 20: 438–445.

Stranahan AM, Arumugam TV, Cutler RG, Lee K, Egan JM, Mattson MP (2008a) Diabetes impairs hippocampal function through glucocorticoid-mediated effects on new and mature neurons. Nat Neurosci 11: 309-17.

Stranahan AM, Norman ED, Lee K, Cutler RG, Telljohann RS, Egan JM, Mattson MP (2008b) Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats. Hippocampus 18: 1085-1088.

Sumiyoshi M, Sakanaka M, Kimura Y (2006) Chronic intake of high-fat and high-sucrose diets differentially affects glucose intolerance in mice. J Nutr 136: 582-587.

Tabák AG, Herder C, Rathmann W, Brunner EJ, Kivimäki M (2012) Prediabetes: a high-risk state for diabetes development. Lancet 379: 2279–2290

Talbot K, Wang HY, Kazi H, Han LY, Bakshi KP, Stucky A, Fuino RL, Kawaguchi KR, Samoyedny AJ, Wilson RS, Arvanitakis Z, Schneider JA, Wolf BA, Bennett DA, Trojanowski JQ, Arnold SE (2012) Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. J Clin Invest 122: 1316-1338.

Thresher JS, Podolin DA, Wei Y, Mazzeo RS, Pagliassotti MJ (2000) Comparison of the effects of sucrose and fructose on insulin action and glucose tolerance. Am J Physiol 279: 1334-1340.

Trudeau F, Gagnon S, Massicotte G (2004) Hippocampal synaptic plasticity and glutamate receptor regulation: influences of diabetes mellitus. Eur J Pharmacol 490: 177-186.

Turrigiano GG, Leslie KR, Desai NS, Rutherford LC, Nelson SB (1998) Activity-dependent scaling of quantal amplitude in neocortical neurons. Nature 391: 892-896.

Urayama A, Banks WA (2008) Starvation and triglycerides reverse the obesity-induced impairment of insulin transport at the blood-brain barrier. Endocrinology 149: 3592-3597.

van der Heide LP, Remarkers GMJ, Marten PS (2006) Insulin signaling in the central nervous system: learning to survive. Prog Neurobiol 79: 205-221.

Vorhees CV, Williams MT (2006) Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat Protoc 1: 848-858.

Wallum BJ, Taborsky GJ Jr, Porte D Jr, Figlewicz DP, Jacobson L, Beard JC, Ward WK, Dorsa D (1987) Cerebrospinal fluid insulin levels increase during intravenous insulin infusions in man. J Clin Endocrinol Metab 64: 1190-1194.

Wilson JS, Mruthinti S, Buccafusco JJ, Schade, RF, Mitchell MB, Harrell DU, Gulati NK, Stephen L, Miller (2009) Anti-RAGE and A immunoglobulin levels are related to dementia level and cognitive performance. J Gerontol 64: 264–271.

Winocur G, Greenwood CE (1999) The effects of high fat diets and environmental influences on cognitive performance in rats. Behav Brain Res 101: 153-161.

Winocur G, Greenwoond C, Piroli G, Grillo A, Reznikov L, Reagen L, McEwen B (2005) Memory impairment in obese Zucker rats: an investigation of cognitive function in an animal model of insulin resistance and obesity. Behav Neurosci 119: 1389–1395.

Wu A, Molteni R, Ying Z (2003) A saturated-fat diet aggravates the outcome of traumatic brain injury on hippocampal plasticity and cognitive function by reducing brain-derived neurotrophic factor. Neuroscience 119: 365-375.

Xu W, Caracciolo B, Wang H-X, Winblad B, Bäckman L, Qiu C, Fratiglioni L (2010) Accelerated progression from mild cognitive impairment to dementia in people with diabetes. Diabetes 59: 2928–2935.

Yashiro K, Philpot BD (2008) Regulation of NMDA receptor subunit expression and its implications for LTD, LTP and metaplasticity. Neuropharmacology 55: 1081-1094.

Zuo ZF, Wang W, Niu L, Kou ZZ, Zhu C, Wang W, Zhao XH, Luo DS, Zhang T, Zhang FX, Liu XZ, Wu SX, Li YQ (2011) RU486 (mifepristone) ameliorates cognitive dysfunction and reverses the down-regulation of astrocytic N-myc downstream-regulated gene 2 in streptozotocin-induced type-1 diabetic rats. Neuroscience 190: 156-165.

Figure 1- Evaluation of spatial learning and long-term memory performance of high-sucrose (HSu) diet and control (Cont) rats using Morris water maze task (spatial reference memory version). (A) shows escape latency (s) to the platform over four days of training; (B) shows time spent (%) in the correct quadrant (probe test); (C) shows time spent (%) in the opposite quadrant (probe test); (D) shows latency (s) to platform zone (probe trial); (E) shows the number of crossings in the platform zone (probe test). Data are presented as mean ± SEM of 8 animals per group. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, *versus* Cont group using an unpaired Student's *t*-test. #, $P < 0.05$; ##, $P < 0.01$, *versus* chance level (25% of time) using one-sample *t*-test.

Figure 2 – Evaluation of short-term spatial memory performance of high-sucrose (HSu) diet group and control (Cont) rats using a modified Y-maze task. A training trial was performed to test exploratory capacity: (A) shows the total number of entries; (B) shows entries (%) in both Start and Other arms; (C) shows time spent (%) in both Start and Other arms. The same parameters were analyzed during test trial: (D) shows the total number of entries; (E) shows entries (%) in Start, Other and Novel arms; (F) shows time spent (%) in Start, Other and Novel arms. Data are presented as mean ± SEM of 8 animals per group. *, $P < 0.05$; **, $P < 0.01$, *versus* Cont group using an unpaired Student's *t*-test. #, $P < 0.05$, *versus* chance level (33.3% of time or entries) using one-sample *t*-test.

Figure 3 - Hippocampal IR-β (A), IRS-1 (B) and IRS-1 pSer (636/639) (C) levels from high-sucrose (HSu) diet and control (Cont) rats (measured by Western blot). High-sucrose decreased IR-β levels (A) but IRS-1 (B) and IRS-1 pSer (636/639) (C) levels remained unchanged in the hippocampus. Data are presented as mean percentage of control ± SEM of 5 animals per group. *, $P < 0.05$, **, $P < 0.01$, *versus* Cont group using an unpaired Student's *t*-test.

Figure 4 - Hippocampal GluA1 (A), GLUN1 (B), PSD-95 (C), synaptophysin (D) and GR (E) levels from high-sucrose (HSu) diet and control (Cont) rats (measured by Western blot). High-sucrose increased both GluA1 (A) and GLUN1 (B) levels but PSD-95 (C) and synaptophysin (D) levels remained unchanged, whereas GR density was decreased in the hippocampus. Data are presented as mean percentage of control ± SEM of 5 animals per group. *, $P < 0.05$, *versus* Cont group using an unpaired Student's *t*-test.

Figure 1

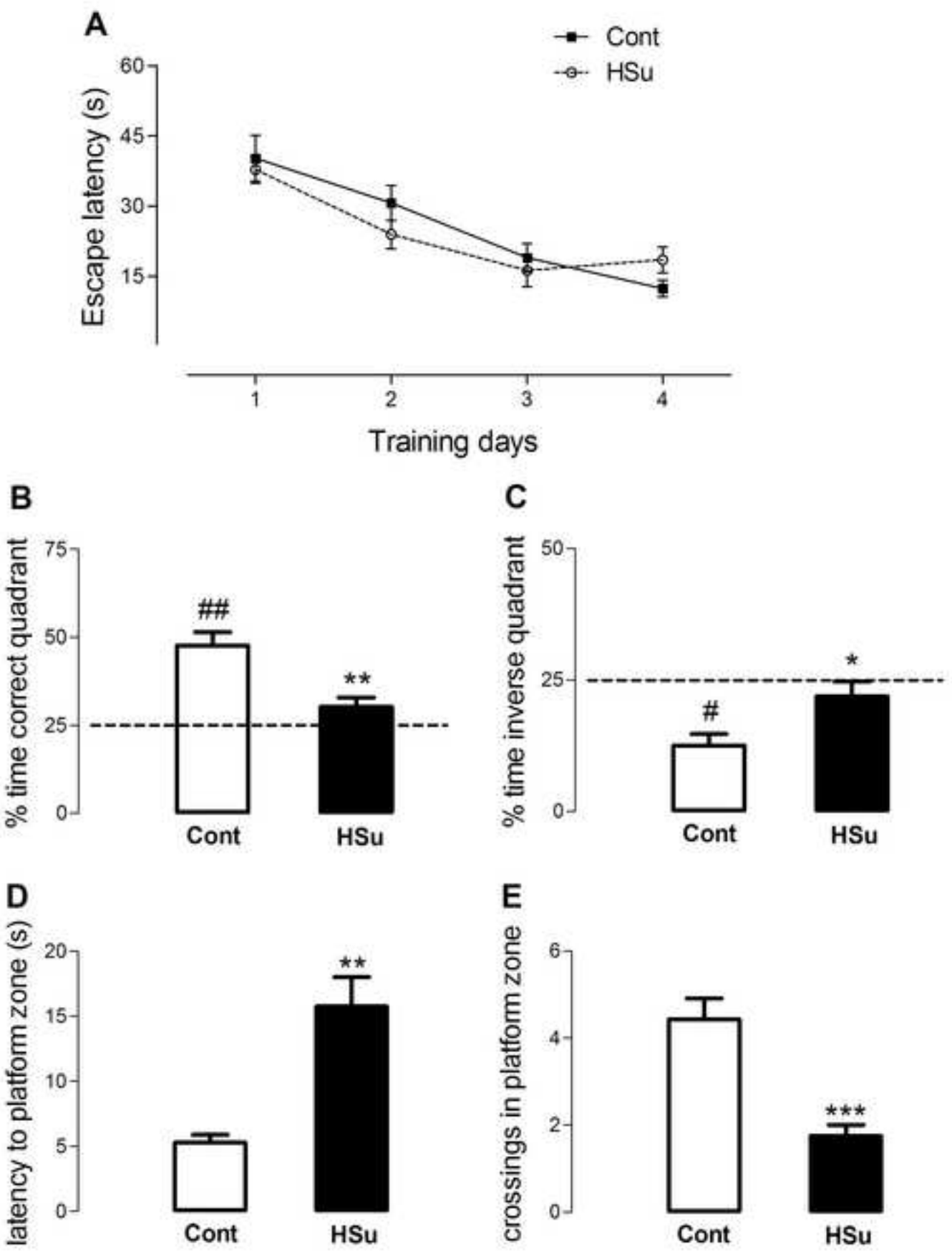


Figure 2

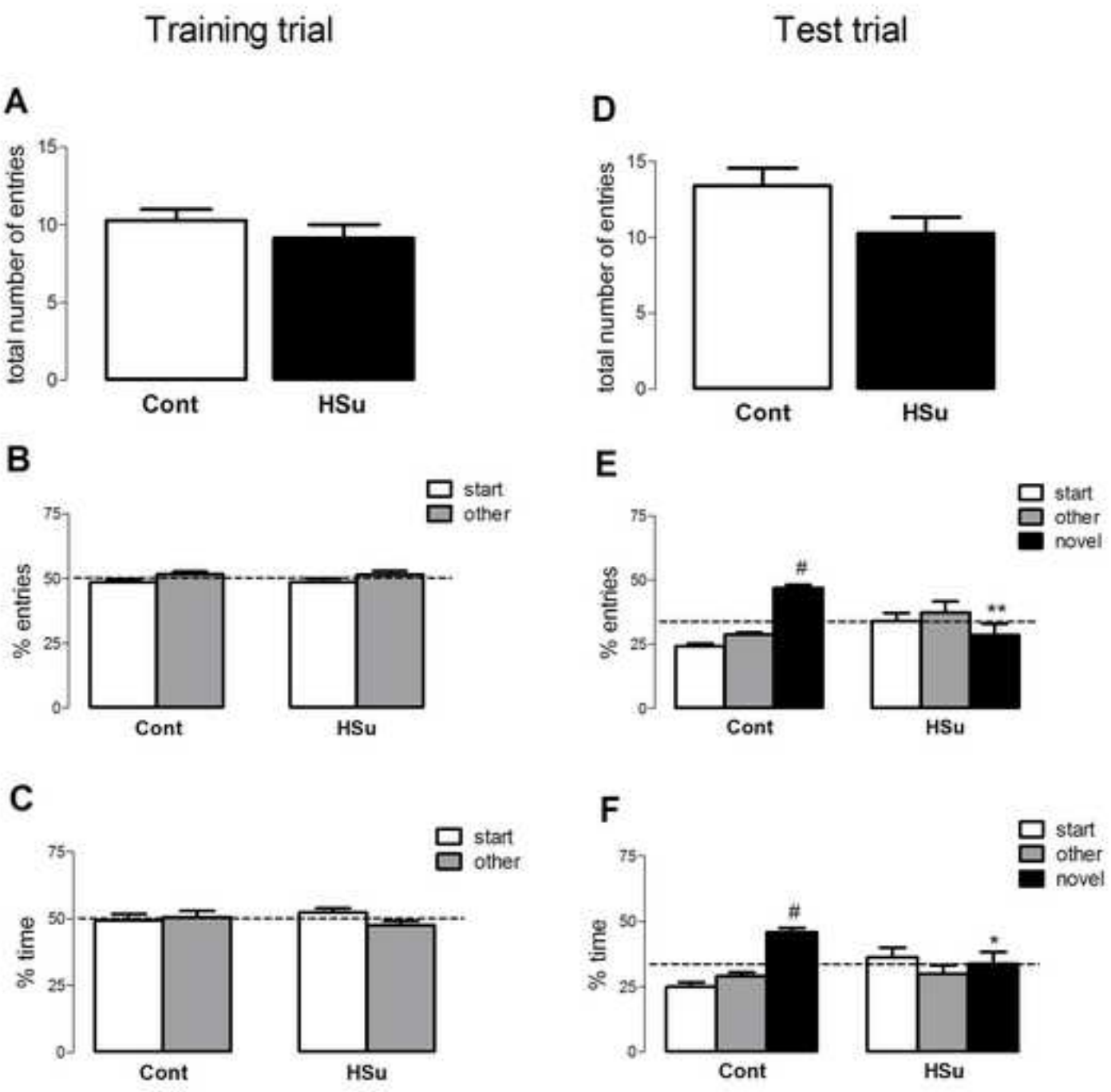


Figure 3

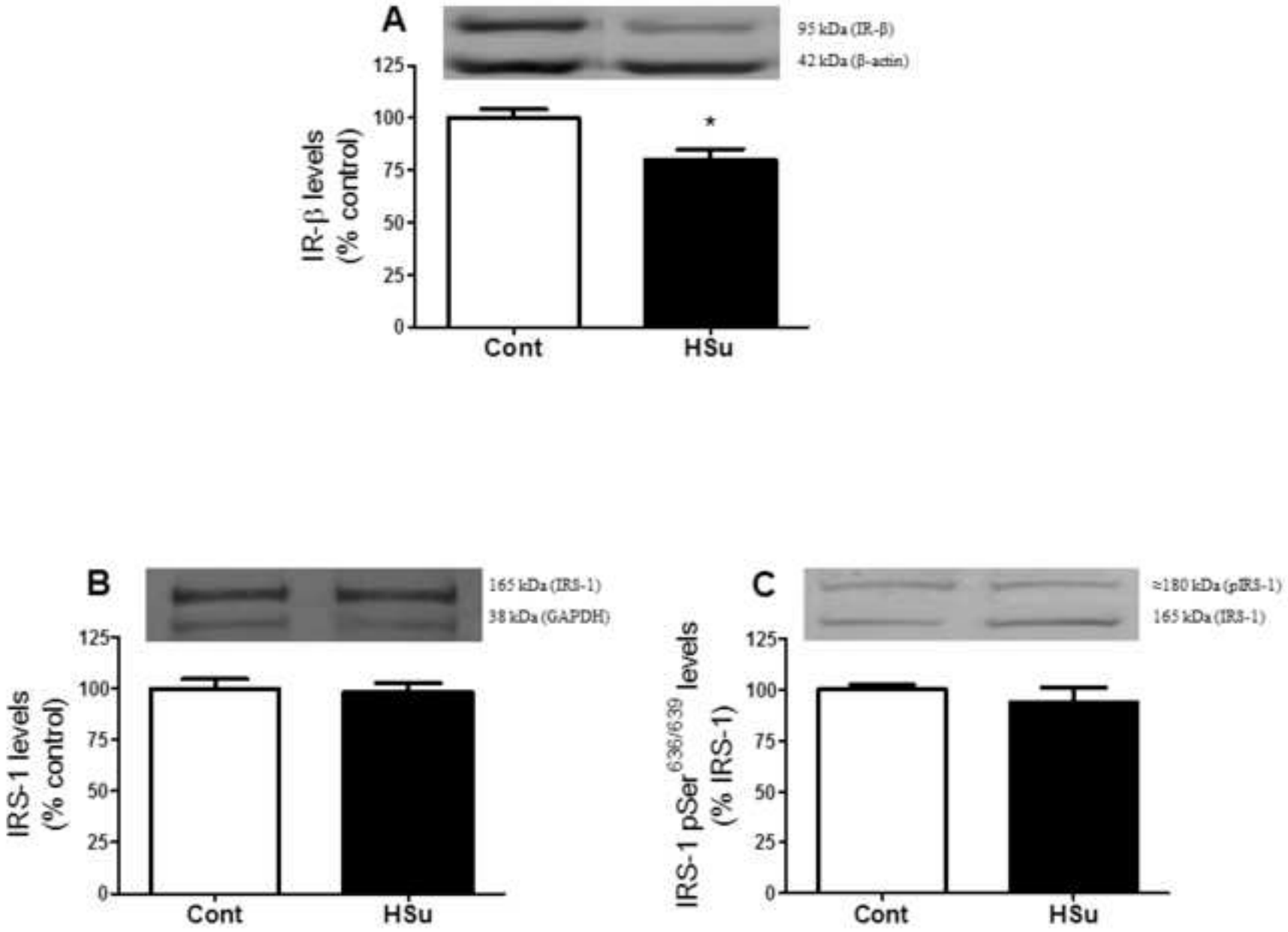
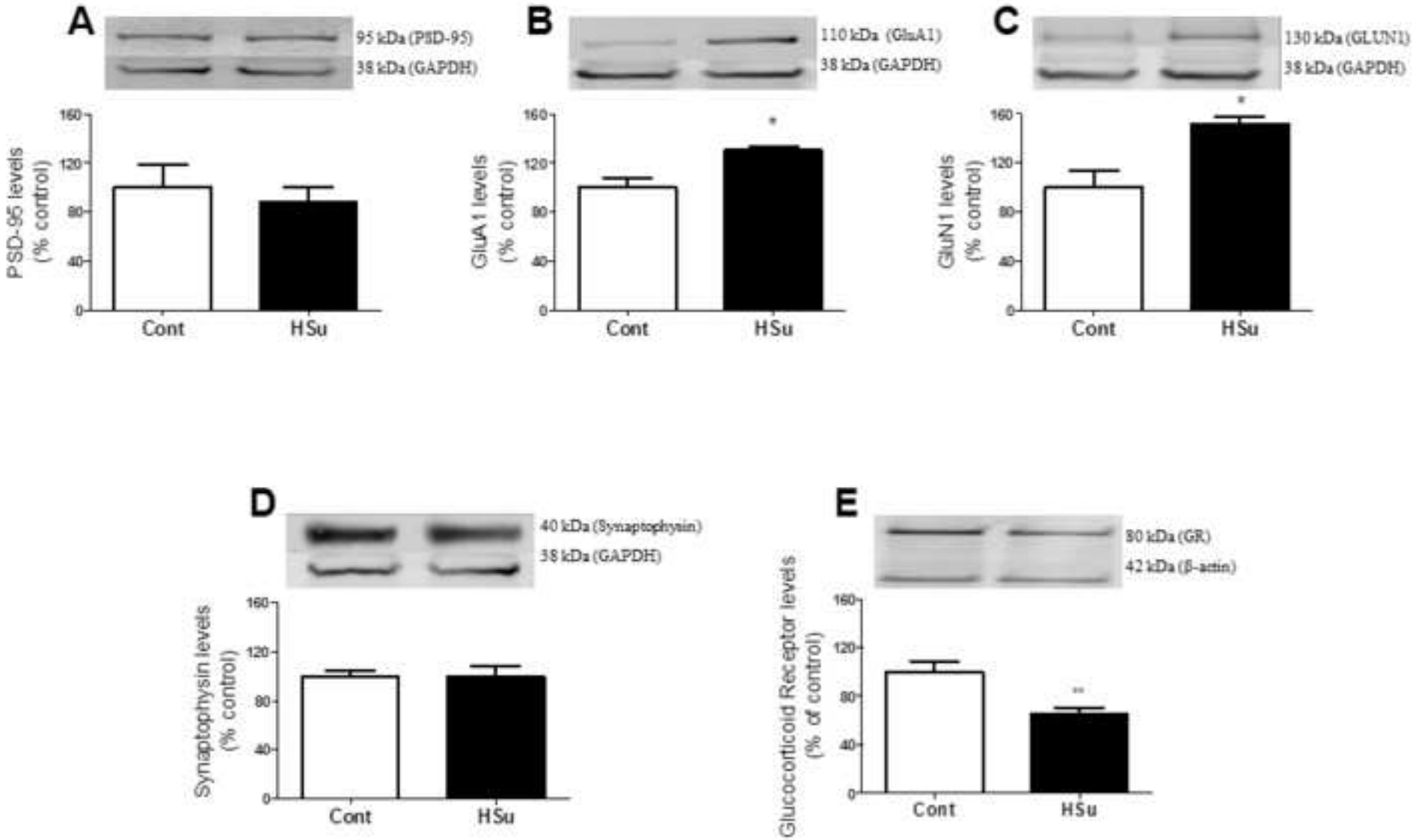


Figure 4



Tables

Table 1: Evaluation of biochemical peripheral parameters of high-sucrose (HSu) diet group and control (Cont) rats.

Parameters	**Cont**	**HSu**
Total Food Consumption (g)	1375 ± 2.9	519 ± 0.7***
Total Drink Consumption (mL)	1833 ± 4.3	2878 ± 4.0***
Body weight (g)	421.0 ± 24.5	421.8 ± 20.3
Postprandial glycemia (mg/dL)	126.8 ± 13.6	162.9 ± 26.5*
Fasting glycemia (mg/dL)	96.7 ± 4.5	102.9 ± 7.0
Fasting Insulin levels (µg/L)	3.7 ± 1.8	10.8 ± 1.0***
Triglyceride levels (mg/dL)	68.1 ± 26.3	143.1 ± 65.7*
Cholesterol levels (mg/dL)	63.7 ± 2.5	58.2 ± 9.7
HbA1c (%)	3.7 ± 0.1	4.0 ± 0.2*
HOMA-IR	$2.7x10^{-5}$±1.5 $x10^{-5}$	$8.0\ x10^{-5}$ ± $9.0\ x10^{-6}$**
Glucose AUC-GTT (mg/dL/120 min)	$2.1\ x10^{4}$ ± $1.0\ x10^{3}$	$2.6\ x10^{4}$ ± $3.8\ x10^{3}$*
Glucose ITT 120 min (mg/dL)	37.2 ± 7.0	63.9 ± 14.8**
Corticosterone levels (µg/mL)	0.541 ± 0.020	0.569 ± 0.041

Data are expressed as mean ± SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ compared with Cont animals.

Table 2**:** Hippocampal oxidative stress/inflammation markers from high-sucrose (HSu) diet and control (Cont) groups.

Parameter	Markers	Cont	HSu	*P value*
Astrogliosis	GFAP	100 ± 9	107± 14	0.6765
	GS	100± 6	102± 8	0.8516
Inflammation markers	TNF-α	100 ± 7	107 ± 2	0.3257
Oxidative stress	RAGE	100 ± 11	107± 16	0.7196
Lipid peroxidation	HNE	100± 8	103± 10	0.8235

Data represent mean percentage of control ± SEM.

Highlights

- Short- and long-term spatial memory deficits are present in prediabetic rats.
- Memory deficits are concurrent with increases of GluA1 and GLUN1 hippocampal levels.
- Prediabetic rats display decreased hippocampal IR-β density.
- Memory deficits are concurrent with decreased GR hippocampal levels.