[Frontiers In Bioscience, Landmark, 24, 291-302, Jan 1, 2019]

Amelioration of prediabetes-induced changes of dendritic structural plasticity

Feiyan Fan[1], Jing Qi[1], Wenshi Wang[2], Nan Liu[1], Hui Liu[1], Xiaoshan Xu[1], Xin Wang[3], Yanyang Tu[1,3], Wen Wang[4], Jianfang Fu[5]

[1]Department of Experimental Surgery, Tangdu Hospital, The Fourth Military Medical University, Xi'an, 710038, Shanxi, China, [2]HemaCare Corporation, 15350 Sherman Way, Van Nuys, CA 91504, California, USA, [3]Department of Neurosurgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, 02215, Massachusetts, USA, [4]Department of Radiology, Tangdu Hospital, The Fourth Military Medical University, Xi'an, 710038, Shanxi, China, [5]Department of Endocrinology, Xijing Hospital, The Fourth Military Medical University, Xi'an, 710032, Shanxi, China

TABLE OF CONTENTS

1. ABSTRACT

Accumulating evidence suggests that the diabetes-induced cognitive dysfunction can be alleviated when exposed to the enriched environment. However, the impact of the changes of the hippocampal plasticity on the cognitive decline and the possible effect of an enriched environment in prediabetes are still not clearly documented. To explore the effect of enriched environment for prediabetes-induced changes of dendritic structural plasticity in hippocampus pyramidal and cognitive deficits, the praxiology experiments for evaluating of anxiety, spatial learning and memory of prediabetic Wistar were performed, and then the dendritic spine density was assessed in the hippocampal CA1 pyramidal neuronal region. The prediabetic rats demonstrated a hyper-anxiety like behavior and significantly decreased spatial learning abilities and memory deficits. Exposing prediabetic rats to an enriched environment appeared to significantly mitigate the above changes in a time-dependent manner. The enriched environment also restored the density of the hippocampal dendritic spine which was significantly reduced in prediabetic

rats. We found that the enriched environment was beneficial in overcoming the prediabetes-induced cognitive disorders and diminished dendritic plasticity of hippocampus pyramidal.

2. INTRODUCTION

Diabetes mellitus, a systemic condition characterized by impaired blood glucose and insulin levels, can cause changes in the structure and function of various tissues and organs including the brain, which is manifested as cognitive dysfunction (1,2). Diabetes induced hippocampus atrophy, Aβ accumulation in hippocampus and cortex, synaptic deletion, and decreased learning and memory capacity has been depicted in diabetic models (3). Another similar life style associated systemic condition, referred to as prediabetes,the subtle balance between glucose and insulin has been thrown off kilter. As recently reviewed by Buysschaert and Bergman (4), the diagnosis of a prediabetic condition, with its symptoms of impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT), and insulin resistance is vital (5). The exact cause for occurrence of prediabetes is not exactly known, however, similar risk factors associated with Tyep 2 Diabetes Mellitus (T2DM), genetics, obesity, sedentary life style, diet and so on are observed related with the prediabetes condition as well. Furthermore, epidemiology data claimed that prediabetes was indeed a well-known risk factor for T2DM (6), and shared similar complications with T2DM, including early forms of nephropathy, chronic kidney disease, small fiber neuropathy, diabetic retinopathy, and cognitive disorders including dementia (5, 7).

Diabetes-induced cognitive dysfunction is a multi-factor, multi-link complex pathological process, which is at the intersection of neurology and endocrinology (8, 9). The clinical and pathologic studies have shown that cognitive dysfunction is associated with atrophy and necrosis of hippocampal and forehead neurous and undergone hypertrophy of amygdala neurons (10). The magnetic resonance imaging of the brain of T2DM patients has confirmed the atrophy in their hippocampus and amygdala, compared to normal subjects (11). Several lines of evidence suggest that those with prediabetes, when compared with a normoglycemic population, are also found to be at an increased risk of cognitive decline (6, 12). In fact, a study in prediabetic mice has demonstrated the association of the cognitive decline with reduced hippocampal microstructure and volume (13). However, more studies are essential to establish such an association.

Therefore, the current treatment of cognitive dysfunction caused by diabetes is still limited and the effect is not very obvious. Depending on the degree of cognitive decline, the mild cognitive dysfunction caused by diabetes is dominated by controlling diabetes through drugs and diet adjustment, and cognitive function training, while moderate to severe cognitive dysfunction are treated with dementia drugs. Studies have shown that the enriched environment (EE) has a certain effect on the recovery of diabetes-induced cognitive dysfunction. Enriched environment can be operationally defined as an experimental strategy in which several research subjects, typically rodents, are exposed to multiple objects of various shapes and size in a vast living space. Exposure to an enriched environment can increase the dentate gyrus (DG) volume by inducing morphological changes, such as increasing hippocampal thickness, enhancing dendritic neuronal branching, and upregulating the number of glial cells (14, 15). Furthermore, studies have shown that the enriched environment can also increase the hippocampal neurons CA1 dendritic density and DG in the anterior ventricular area (16, 17). However, it is unclear whether the enriched environment can alleviate the cognitive impairment induced by prediabetes.

Based on the above knowledge, we hypothesized that the insulin-resistance mediated prediabetes condition can induce cognitive decline while inducing changes in hippocampal pyramidal dendritic structural plasticity; and an enriched environment may alleviate these changes. This hypothesis was proved in the present study using an established prediabetic Wistar rat animal model.

3. MATERIALS AND METHODS

3.1. Experimental animals

Thirty male Wistar rats and rat feed were obtained from the Fourth Military Medical University Animal Center (Xi'an, Shanxi, China). All the animals went through a period of adaptation for 3 days, under the following conditions: free access to food (regular diet) and water, light and dark cycle of 12 h, room temperature 20 ~ 26 and humidity 40% ~ 70%. On the fourth day, a group of 6 rats were randomly assigned to naive control group and were continued on a regular diet of food and water. The rest of the animals were treated with drinking water containing 35% sucrose, deemed high sucrose (HSu) group, and were exposed to an enriched environment every day for 0 h (HSu+0h), 2 h (HSu+2h), 4 h (HSu+4h), 6 h (HSu+6h), which formed the four experimental groups. The enriched environment cages (52cm×37cm×22cm) consisted of two running wheels, a platform, three tunnels, a variety of toys, and nesting material. The standard environment cages (32cm×20cm×15cm) only consisted of nesting material. Food and beverage consumption was monitored for each group throughout the experiment. The weight of each rat was measured weekly, along with their fasting serum glucose and

fasting serum insulin level. Morris water maze test was started on day 64and continued for 6 days. Behavioral experiments, including open field test and elevated plus maze test were conducted on day 70. On day 71, post euthanasia, the brains of the animals were subject to morphological studies.

3.2. Biochemical Analysis

The fasting blood sugar (FBS) was determined by using the blood sugar meter and test strips each week. The fasting serum insulin (FINs) was determined by ELISA kit. The HOMA-IR=fasting serum glucose ×fasting serum insulin/22.5.the values used were obtained after an overnight fasting period. All of the parameters were performed according to the manufacturer's instructions. The blood samples and viscera organs were stored at -80°C in the refrigerator for future research.

3.3. Open field test

Following a previously reported method (18), the rats' behavior in a different environment was evaluated by subjecting the animal to an open field task. Briefly, the experimental rats were placed in a dark open field observation box, of 1.5.m x 1.5.mx 0.6.m in diameter, and were given an incubation period of 10 seconds. Using a camera placed above the open field, the activity of the animals was recorded for 15 min. Post recording, the videos were analyzed for the following behavioral indicators: the total distance of movement and total time of stay in the center.

3.4. Elevated Plus Maze test

The anxiety level of the rats was assessed through elevated plus maze (EPM) task, following the method reported by Komada *et al.* (19). Briefly, the experimental rats were placed on the central platform of EPM, and the actions were recorded for 5 min (20). post incubation time of 5 seconds the videos were assessed for the following anxiety indicators: number of entries into the open arms and the time spent in the open arms.

3.5. Morris Water Maze test

Following a previously reported method (21), the spatial learning and memory ability of the rats were assessed through Morris water maze (diameter 100 cm, water depth 35 cm, platform diameter 33 cm) test. The system was equipped with a roof mounted digital camera 180 cm above the water surface to monitor the animal behavior.

3.6. Tissue preparation and Golgi staining

Experimental rats were euthanized by decapitation under deep pentobarbital anesthesia (50 mg / kg; intraperitoneally) and the brain tissue was removed immediately. Using the FD Golgi staining kit (FD Neurotechnologies, Eliot, MD, USA), the tissue was processed, coronal sections from Bregma-3.0.0 to Bregma 3.6.0, of 100 µm in thickness, were obtained and stained as per the manufacturer instructions. The stained tissue was scanned for dendritic segments using a phase contrast microscope (DN-107T, AS ONE, Osaka, Japan), and fully impregnated and separated 20 CA1 neuronal cell body with no apparent severed dendrites identified in each group for imaging. The images were captured at 25X, and the density of the dendritic spine, defined as the first branch of the dendritic neurons or the main dendrites of the bipolar neurons within a random segment of 10 µm in length, was assessed using NeuroExplorer software (MicroBrightField, Williston, VT, USA). Sholl's analysis was used to determine any changes in spatial distribution of dendritic spines relative to the cell body (22). Seven 50-µm-wide spherical bins were formed around each cell body, and spine density within each bin was averaged for each treatment group. For statistical comparison, spine density at dendrite branch locations within 50–150 µm (proximal bins) and 200–350 µm (distal bins) from the cell body were pooled and compared across treatment groups.

3.7. Statistical analysis

Data in each test was expressed as mean ± standard deviation. Statistical analysis was performed using SPSS 17.0. software, and Origin Pro 8.1. software was used for the drawing of charts and graphs. The variation in the physiological parameters, including body weight, FBS values, fasting insulin levels and HOMA-IR values, were analyzed with unpaired Student's *t*-test. The group difference in behavioral performance was assessed with two-way ANOVA, while the Single factor analysis of variance (One-Way, ANOVA) was employed for dendritic density analysis. The difference was considered significant when p-value was less than 0.0.5.

4. RESULTS

4.1. The prediabetic state of the experimental rats

During the 9 weeks of treatment, the HSu rats, including those exposed to the enriched environment, showed no changes in their body weight and fasting blood glucose (FBS) levels compared to the control animals. However, HSu consumption induced an elevation on postprandial blood glucose (p<0.0.5) (Table 1 and 2) while their fasting insulin levels spiked as early as 1 week after starting 35% sucrose diet, and continued to rise from then onwards (Table 3), thus demonstrating a significant hyperinsulinemia (p<0.0.5) in HSu group, compared to control animals. The corresponding increase in the HOMA-IR values also reached statistical significance (p<0.0.5) in Hsu rats compared to control rats (Table 4). Exposing the

Table 1. Total water consumption corresponding to the observed hyperinsulinemia in high sucrose fed male Wistar rats

Group Time	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
Total water consumption (mL/day)										
Naive	60.4.±2.5.	67.7.±3.8.	76.3.±2.9.	87.2.±2.6.	99.5.±3.1.	113.3.±2.3.	128.3.±2.4.	145.4.±3.2.	162.8.±4.1.	180.5.±3.9.
Hsu+0h	59.6.±3.4.	70.7.±2.7.	93.9.±3.6.	109.2.±4.2.*	129.5.±2.8.*	155.2.±2.9.*	183.7.±1.7.*	213.2.±3.5.*	246.7.±4.1.*	283.3.±4.3.*
Hsu+2h	58.6.±3.6.	68.3.±1.9.	82.6.±4.8.	95.3.±3.6.	113.5.±4.2.	138.2.±2.7.*	164.7.±4.4.*	297.3.±2.6.*	230.8.±3.6.*	265.7.±2.5.*
Hsu+4h	59.5.±2.2.	68.2.±2.8.	79.4.±3.7.	91.6.±2.5.	106.5.±1.8.	125.4.±3.6.	140.8.±3.5.	160.9.±2.7.	190.8.±4.5.*	224.4.±3.6.*
Hsu+6h	60.6.±2.4.	69.7.±4.2.	79.6.±5.1.	90.2.±3.1.	102.6.±2.4.	117.3.±3.7.	136.9.±2.3.	155.2.±3.5.	179.4.±4.8.	201.6.±4.3.

Comparison of the total water consumption between group (Hsu+0h, Hsu+2h,Hsu+4h, Hsu+6h) and Naive group. The data are expressed as mean±standard error and the statistical analysis were performed by Students t-test. *p< 0.0.5.

Table 2. Postprandial blood level observed in male Wistar rats fed with high sucrose for 9 weeks

Group Time	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
Postprandial blood glucose level (mg/dL)										
Naive	126.2.±3.3.	127.3.±4.2.	126.8.±3.4.	125.9.±4.5.	127.3.±3.2.	127.2.±5.1.	126.9.±3.5.	128.1.±4.8.	126.8.±4.9.	127.1.±5.7.
Hsu+0h	127.5.±4.6.	128.1.±4.5.	129.3.±4.7.	132.8.±5.5.	135.4.±5.4.	140.6.±4.2.	145.6.±3.8.*	151.5.±4.7.*	157.8.±5.3.*	163.9.±4.2.*
Hsu+2h	126.6.±3.8.	127.5.±5.5.	128.9.±3.4.	131.1.±4.8.	134.2.±4.3.	138.7.±4.6.	142.3.±3.3.	146.4.±5.4.*	150.9.±5.2.*	155.6.±3.5.*
Hsu+4h	127.3.±4.5.	127.5.±3.5.	128.8.±4.5.	129.9.±3.5.	130.5.±4.9.	133.5.±4.7.	135.6.±4.7.	139.4.±4.8.	140.2.±3.6.	140.8.±4.3.
Hsu+6h	127.1.±4.6.	126.9.±4.5.	127.6.±6.1.	127.8.±4.2.	128.5.±4.7.	128.9.±4.5.	129.3.±3.8.	129.5.±4.4.	129.8.±5.3.	131.2.±4.4.

Comparison of the postprandial blood glucose level between group (Hsu+0h, Hsu+2h,Hsu+4h, Hsu+6h) and Naive group. The data are expressed as mean±standard error and the statistical analysis were performed by Students t-test. *p< 0.0.5.

Table 3. Hyperinsulinemia observed in male Wistar rats fed with high sucrose for 9 weeks

Group Time	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
Fasting Insulin level (µg/L)										
Naive	3.2.±1.1.	3.6.±0.9.	3.8.±1.5.	3.4.±0.7.	3.9.±0.5.	3.6.±1.7.	3.7.±1.3.	3.9.±1.0.	3.8.±0.4.	3.7.±1.3.
HSu+0h	3.4.±1.7.	6.7.±1.7.*	4.2.±1.8.	6.3. ±2.0.*	7.4.±2.3.*	9.1.±1.9.*	9.2.±0.4.*	8.7.±1.7.*	10.1.±1.1.*	10.9.±0.3.*
HSu+2h	3.4.±2.9.	5.9.±1.7.	4.9.±2.1.	7.1.±1.8.*	7.0.±3.1.*	9.0.±2.6.*	8.8.±2.4.*	9.1.±2.1.*	9.8.±3.0.*	11.5.±3.5.*
HSu+4h	3.4.±2.4.	4.6.±2.9.	4.0.±3.8.	7.5.±4.6.*	6.9.±4.2.*	7.7.±2.4.*	9.1.±3.7.*	10.2.±2.8.*	10.0.±1.8.*	9.5.±4.6.*
HSu+6h	3.4.±2.2.	7.2.±3.7.*	5.8.±3.3.	5.3.±2.6.	7.9.±1.1.*	5.9.±0.3.*	8.9.±1.2.*	9.0.±2.6.*	9.9.±3.8.*	10.5.±1.4.*

* Comparison of the fasting insulin level between group (Hsu+0h, Hsu+2h,Hsu+4h, Hsu+6h) and Naive group. Data are expressed as mean±standard error and the statistical analysis were performed by Students t-test. *p< 0.0.5.

Table 4. HOMA-IR values corresponding to the observed hyperinsulinemia in high sucrose fed male Wistar ats

Group Time	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
HOMA-IR values										
Naive	$2.1\times10^{-5}\pm 5.2\times10^{-6}$	$2.5\times10^{-5}\pm 1.8\times10^{-5}$	$2.1\times10^{-5}\pm 9.3\times10^{-6}$	$2.4\times10^{-5}\pm 7.6\times10^{-6}$	$2.8\times10^{-5}\pm 1.0\times10^{-5}$	$2.5\times10^{-5}\pm 8.3\times10^{-6}$	$2.9\times10^{-5}\pm 1.3\times10^{-5}$	$2.9\times10^{-5}\pm 6.7\times10^{-6}$	$2.5\times10^{-5}\pm 1.1\times10^{-5}$	$2.5\times10^{-5}\pm 1.5\times10^{-5}$
HSu+0h	$2.9\times10^{-5}\pm 1.8\times10^{-5}$	$4.2\times10^{-5}\pm 1.1\times10^{-5}$	$3.9\times10^{-5}\pm 7.3\times10^{-6}$	$5.7\times10^{-5}\pm 1.0\times10^{-5}$	$4.9\times10^{-5}\pm 1.5\times10^{-5}$	$8.8\times10^{-5}\pm 1.9\times10^{-5*}$	$6.9\times10^{-5}\pm 7.9\times10^{-6*}$	$7.4\times10^{-5}\pm 8.5\times10^{-6*}$	$8.1\times10^{-5}\pm 1.0\times10^{-5*}$	$7.9\times10^{-5}\pm 1.5\times10^{-5*}$
HSu+2h	$2.5\times10^{-5}\pm 1.9\times10^{-5}$	$2.8\times10^{-5}\pm 2.5\times10^{-5}$	$5.1\times10^{-5}\pm 1.0\times10^{-5}$	$3.8\times10^{-5}\pm 2.7\times10^{-5}$	$6.9\times10^{-5}\pm 1.4\times10^{-5*}$	$7.6\times10^{-5}\pm 1.8\times10^{-5*}$	$8.3\times10^{-5}\pm 6.2\times10^{-6*}$	$7.5\times10^{-5}\pm 2.1\times10^{-5*}$	$9.0\times10^{-5}\pm 1.2\times10^{-5*}$	$9.1\times10^{-5}\pm 4.1\times10^{-5*}$
HSu+4h	$3.0\times10^{-5}\pm 0.5\times10^{-6}$	$3.9\times10^{-5}\pm 4.0\times10^{-6}$	$3.2\times10^{-5}\pm 2.2\times10^{-5}$	$5.7\times10^{-5}\pm 1.1\times10^{-5}$	$7.0\times10^{-5}\pm 1.2\times10^{-6*}$	$8.9\times10^{-5}\pm 3.5\times10^{-6*}$	$7.9\times10^{-5}\pm 1.5\times10^{-5*}$	$8.0\times10^{-5}\pm 1.6\times10^{-6*}$	$7.9\times10^{-5}\pm 2.3\times10^{-5*}$	$8.4\times10^{-5}\pm 0.7\times10^{-5*}$
HSu+6h	$2.6\times10^{-5}\pm 1.5\times10^{-5}$	$3.0\times10^{-5}\pm 9.3\times10^{-6}$	$4.5\times10^{-5}\pm 1.3\times10^{-5}$	$6.5\times10^{-5}\pm 8.8\times10^{-6}$	$6.4\times10^{-5}\pm 1.8\times10^{-6}$	$7.0\times10^{-5}\pm 1.6\times10^{-5*}$	$7.9\times10^{-5}\pm 9.4\times10^{-6*}$	$8.0\times10^{-5}\pm 1.9\times10^{-5*}$	$7.6\times10^{-5}\pm 2.0\times10^{-5*}$	$7.0\times10^{-5}\pm 1.9\times10^{-5*}$

Comparison of the HOMA-IR values between group (Hsu+0h, Hsu+2h,Hsu+4h, Hsu+6h) and Naive group. The data are expressed as mean±standard error and the statistical analysis were performed by Students t-test. *p< 0.0.5.

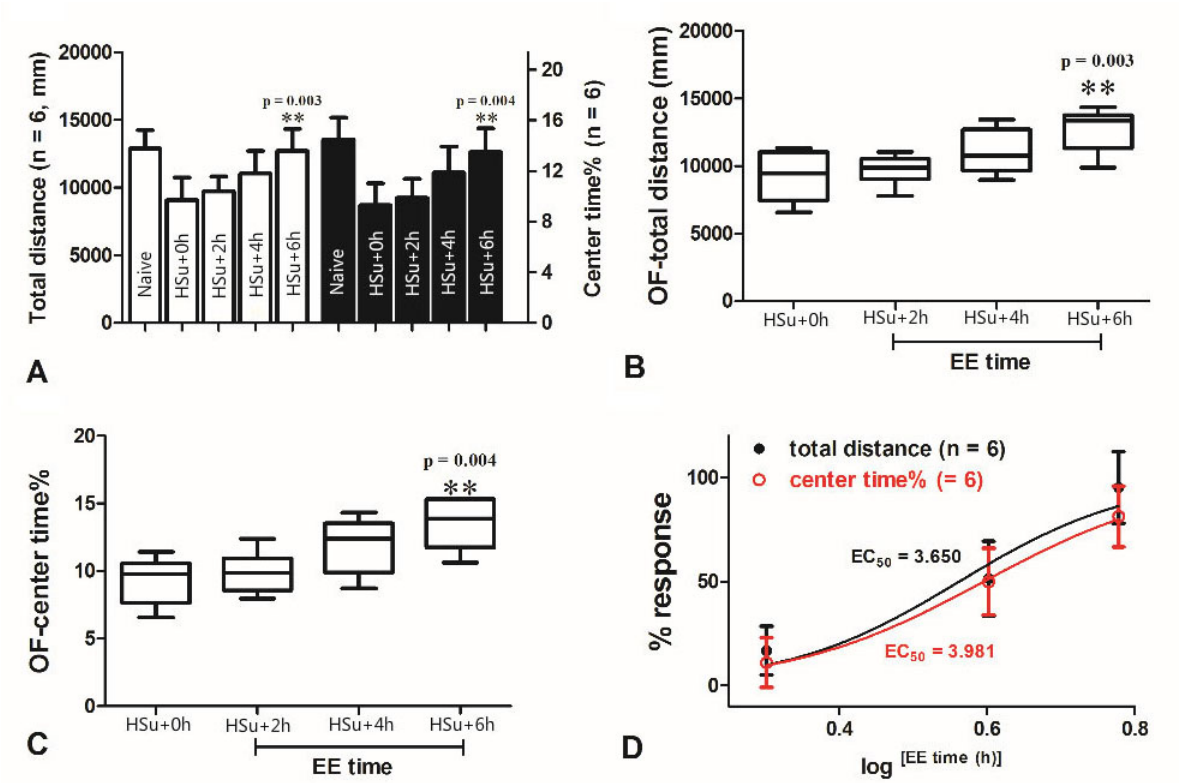

Figure 1. Enriched environment ameliorates prediabetes induced anxietylike behavior in the open field (OF), in a time-dependent manner. (A) indicates the average of the total distance (mm) travelled by study rats in the dark open field box and total time of their stay at the center of the dark open field box; (B) indicates the median of the total distance (mm) travelled by study rats in the dark open field box; (C) indicates the median of the total time of stay by the study rats at the center of the dark open field box; (D) indicates the EC_{50} of the enriched environment for the total distance travelled and total time of stay at the center of the dark open field box; ** p<0.0.1; * p <0.0.5, compared with HSu+0 h.

sucrose fed rats to enriched environment had no effect on the observed hyperinsulinemia (Table 3) and thus HOMA-IR values (Table 4). Considering the fasting normoglycemia and hyperinslinemia, and the HOMA-IR values indicative of insulin resistance, the HSu rats were considered prediabetic before undergoing behavioral and morphological assessments (26).

4.2. Enriched environment ameliorates prediabetes-induced anxiety behaviors in the open field (OF) in a time-dependent manner

In the open field test, the high sucrose fed rats showed a significant decrease in the total distance traveled (p<0.0.1; Figure 1A and 1B) within the dark open field, while also showing a significantly decreased total time of stay at its center (p<0.0.01; Figure 1A and 1C), as compared to the naive rats, demonstrating anxiety-like behavior in these prediabetic rats. However, the prediabetic rats exposed to the enriched environment demonstrated lower anxiety levels, which was directly proportional to the duration of exposure. The HSu+6h group managed to reach statistical significance in terms of both total distance traveled (p=0.0.03; Figure 1A and 1B)) and total time of stay at the center of the box (p=0.0.04; Figure 1A and 1C). We also calculated the EC_{50} of the enriched environment, which was found to be 3.6.50h and 3.9.82h for a total distance traveled and a total duration of time spent at the center of the open field box, respectively.

4.3. Enriched environment ameliorates prediabetes-induced depressive behaviors in the elevated plus maze (EPM) in a time-dependent manner

Similar to the increased anxiety behavior, the prediabetic rats also demonstrated depressive behavior, as demonstrated by a significantly decreased number of entries into (p=0.0.04; Figure 2A and 2B) and the total time of stay (p<0.0.01; Figure 2A and 2C) in the open arm within the elevated plus maze test. The enriched environment, however, could overcome this prediabetes induced depressive behavior in a dose dependent manner. Rats which received enriched environment treatment showed increased entries into open arms and staying there for a longer period of time. The HSu+6h group reached statistical significance in both the number of entries (p=0.0.36; Figure 2A and 2B) and total time of stay (p<0.0.01; Figure 2A and 2C) in the open arms compared to the

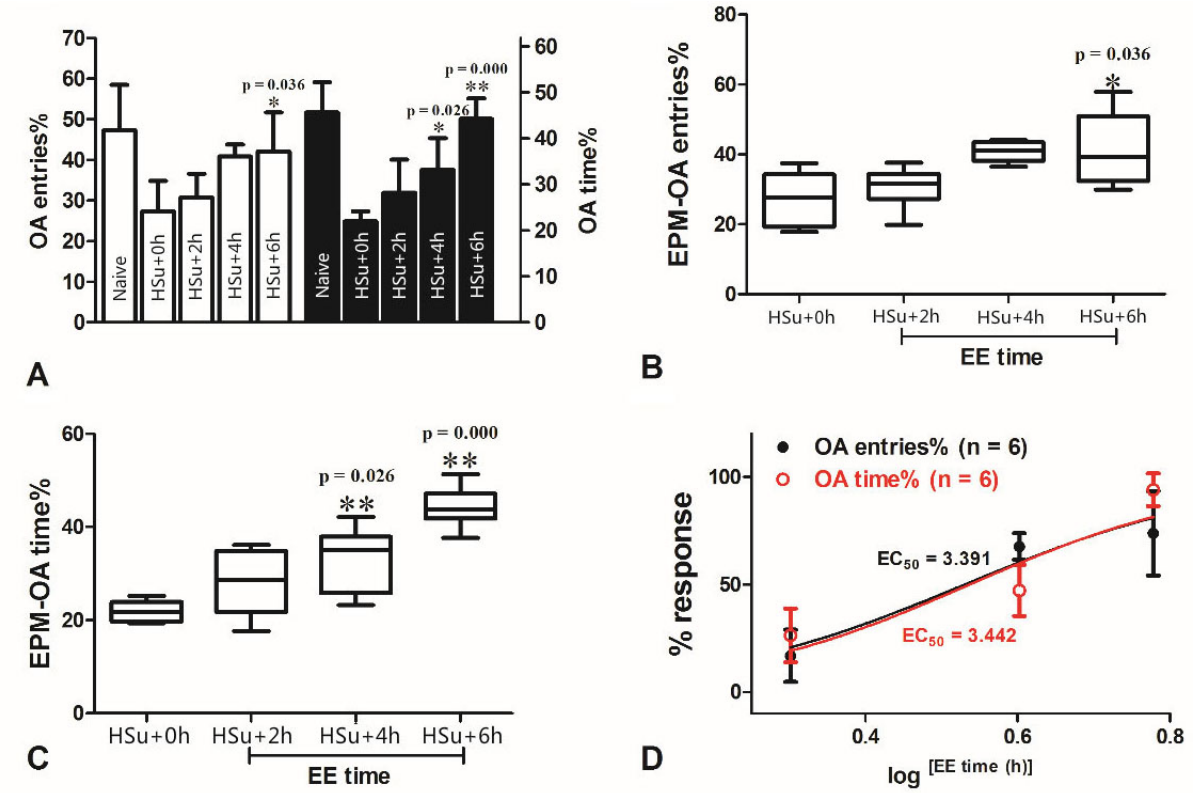

Figure 2. Enriched environment ameliorates prediabetes induced anxietylike behavior in the elevated plus maze (EPM) test, in a time-dependent manner. (A) indicates the average of the percentage (%) of the number of times the study rat enters open arm and the total time of stay in the open arm of the elevated plus maze; (B) indicates the median of percentage (%) of the number of times the study rat enters the open arms of the elevated plus maze (C) indicates the median of the percentage (%) of the total time of stay in the open arm by study rats in the elevated plus maze (D) indicates the EC_{50} of the total number of entries into the open arm of the elevated plus maze and the total time of stay in the same by the study rats; ** p<0.0.1; * p <0.0.5, compared with HSu+0 h.

HSu+0h group, while the HSu+4h group managed to reach significance in terms of total time of stay analysis (p=0.0.26). The EC_{50} of the total number of entries into the open arms of the elevated plus maze and the total time of stay in the same by the study rats was determined to be 3.3.91h and 3.4.42h, respectively.

4.4. Enriched environment ameliorates prediabetes-induced impairment in the spatial learning/memory in the morris water maze (MWM) test in a time-dependent manner

In order to evaluate the effect of enriched environment on impaired spatial learning, a learning test was performed. On the 5th day of MWM training, compared to naive group rats, we observed that the prediabetic rats took a significantly longer path to reach the platform (p<0.0.05), with a corresponding increase in their escape latency (p<0.0.05), demonstrating reduced learning abilities (Figure 3A and 3B). However, the prediabetic rats exposed to an enriched environment showed better learning abilities compared to the HSu+0h group rats, as demonstrated by a significant reduction in the path length to find the platform by the HSu+6h (p<0.0.05) and the HSu+4h (p<0.0.5) group rats, and in escape latency by the HSu+6h (p<0.0.05), the HSu+4h (p<0.0.5) and the HSu+2h (p<0.0.5) group rats (Figure 3A and 3B). Within the target quadrant, the traveled path length was not significantly different among any of the assessed groups of rat.

In order to detect spatial learning and memory ability after training trial, memory test was used to perform probe trial. The high sucrose fed rats spent a significantly lower amount of time in the target quadrant (p<0.0.05), added by a significantly lower number of platform crosses (p<0.0.01), revealing an impaired memory in these prediabetic rats. However, the prediabetic rats exposed to 6 hours of the enriched environment spent significantly more time in the target quadrant (p<0.0.1) with an increased number of platform crossings (p<0.0.1), demonstrating a better memory function compared to the HSu+0h group rats.

4.5. Enriched environment restored the descreased hippocampal pyramidal dendritic structural plasticity in a time-dependent manner

Considering the observed behavioral changes in the high sucrose fed rats, we looked for any changes in the plasticity of the hippocampal

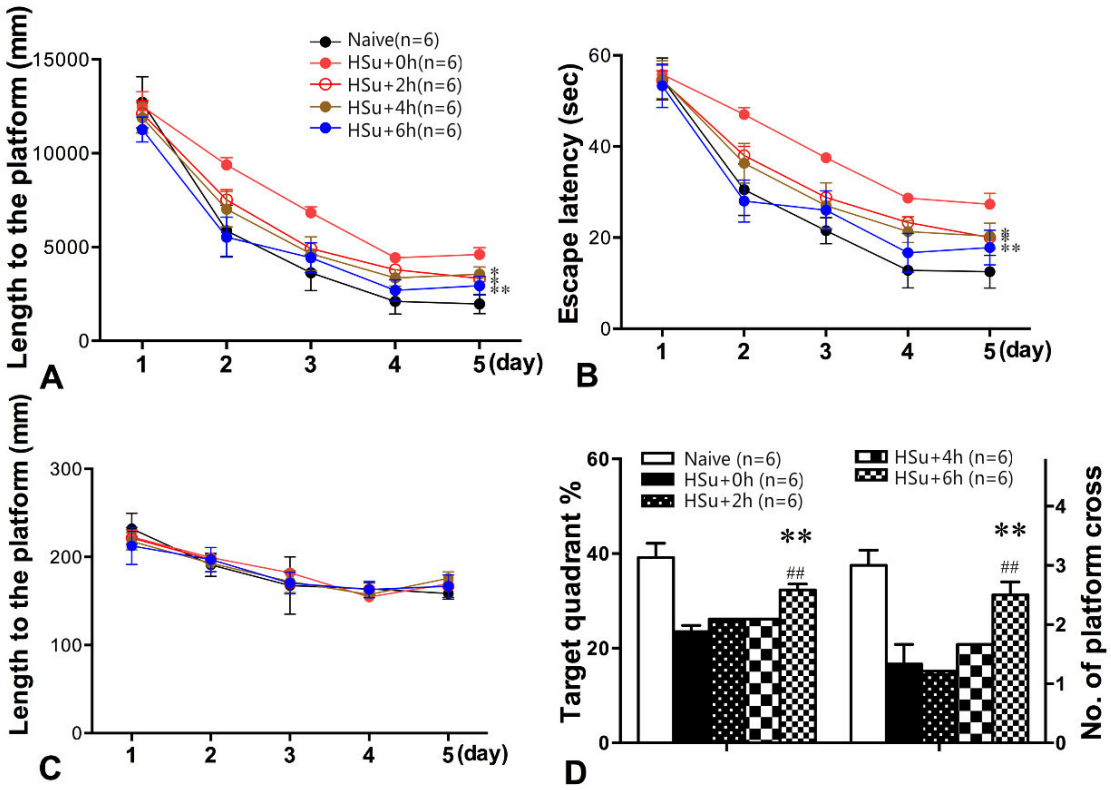

Figure 3. Enriched environment ameliorates prediabetes induced impairment in the spatial learning/memory in a time-dependent manner as demonstrated by the morris water maze (MWM) test. (A) indicates the total distance covered by the study rats to reach the platform; (B) indicates the escape latency of the study rats; (C) indicates the total distance covered by the study rats to reach the platform within the target quadrant; (D) indicates the percentage of time spent in the target quadrant and the number of times the study rats cross the platform zone; ** p<0.0.1; * p<0.0.5, compared with HSu+0 h.

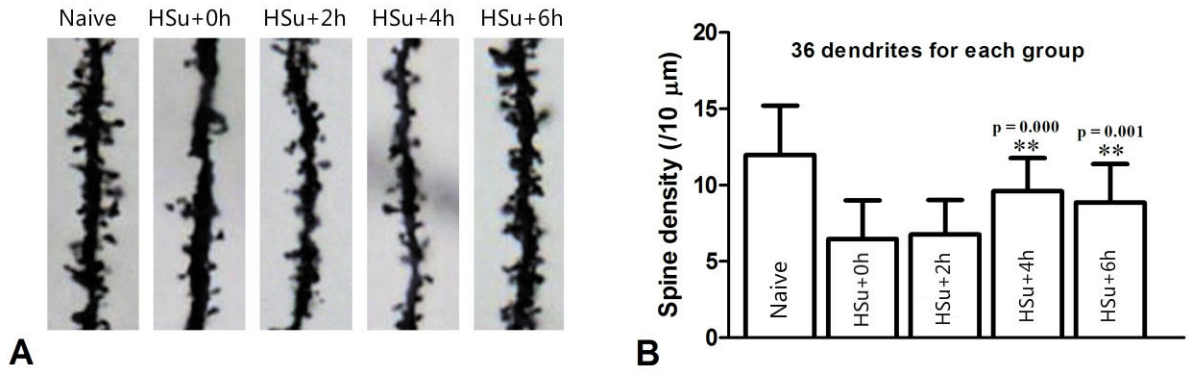

Figure 4. Enriched environment restores abnormal hippocampal pyramidal dendritic structural plasticity in a time-dependent manner. (A) indicates the hippocampal CA1 neuronal dendrites and their dendritic spines; (B) indicates the hippocampal CA1 neuronal dendritic spine density; ** p <0.0.1; * p<0.0.5, compared with HSu+0 h.

pyramidal dendrites. The HSu+0h group rats showed a significant decrease in the dendritic spine density (p<0.0.01), compared to that of the naive group rats (Figure 4A and 4B). Long-term exposure of these prediabetic rats to an enriched environment was able to retain a significantly higher density of dendritic spines compared to the HSu+0h group, especially in the HSu+4h (p<0.0.01) and the HSu+6h (p<0.0.01) groups (Figure 4A and 4B).

5. DISCUSSION

Type-2 diabetes mellitus (T2DM), a life-style related systemic condition, can cause changes in the structure and function of multiple tissues and organs, including the brain resulting in various behavioral and psychological disorders (23). Studies have revealed a high incidence and a high recurrence rate of behavioral disorders in T2DM (24). In diabetic rat models,

the association of the occurrence of anxiety and depression like behavior with impaired spatial learning abilities and diminished memory has been established and extensively studied (25). Research studies have shown that diabetes-induced Aβ aggregation can cause the loss of neuronal synapses and neurons themselves, leading to impaired cognitive function (26). Furthermore, Tan *et al.* found that diabetes and prediabetic states characterized byinsulin resistance, hyperinsulinemia, and hyperglycemia, when present in late middle age, were related to decreased brain volume and lower cognitive performance on executive function and memory tasks (27). It is noteworthy that the cognitive dysfunction, psychological and emotional disorders associated with the development of diabetes are multi-factorial, involving a complex pathogenesis, making it a robust field of research (28).

However, these effects are seldom studied in the prediabetic condition, a high-risk malady for developing T2DM exhibiting a normal fasting blood glucose level, an impaired post prandial blood glucose level, an impaired glucose tolerance and hyperinsulinemia. This was addressed in a current study using a prediabetes rat model, male Wistar rats were fed with a high sucrose diet (35%) for 9 weeks (29).The HSu-diet as a rat model of prediabetes with impaired glucose tolerance and insulin resistance, associated with hippocampal alterations represent some of the early brain complications (30). At the end of the 9 weeks, the prediabetic condition of the study rats was confirmed by increased insulin resistance which was indicated by the significantly increased HOMA-IR index values (31). The behavioral tests were executed on the prediabetic rats and the control rats. In agreement with a recent report, in our open field test, the prediabetic rats ventured less into the central anxiogenic zone, also spending significantly less time whenever they did so, demonstrating a hyper-anxiety like behavior compared to normal control rats (32). This hyper-anxiety like behavior in the rats was further clarified by the elevated plus maze test, where the prediabetic rats preferred to enter and stay in the closed arm as opposed to the open arm, when compared to the normal control rats. Soares E *et al.* (30) showed spatial memory impairment in prediabetic rats, demonstrated by the water maze test. Confirming this report, the prediabetic rats that were challenged with the water maze task in our study showed severe decline in spatial learning and memory abilities, explained by a significant increase in path length and escape latency.

The mechanism by which enriched environment ameliorates behavioral deficits and reduced spine density remain unclear. In our study, the cognitive decline induced by HSu-diet correlated with the changes in the plasticity of hippocampal dendritic spines, as observed where there was a severe decrease in the dendritic spine density. This result was supported by Stranahan AM *et al.* in a T2DM rat model (33). Many lines of evidence support that enriched environment increases the availability of trophic factors, which in turn mediate changes to neurons and their supporting network (34). Several attempts have been made to prove that BDNF is one of the key factors involved in the brain protection incluced by enriched environment (35,36). Such a decrease in hippocampal dendritic spine density has been previously attributed to the decrease in the expression of PSD-95 protein in a prediabetic mice model (13). Furthermore, Soares E. *et al.* (30) studied the prediabetes induced biochemical changes in the hippocampus of a Wistar rat model. The results revealed an alternation in hippocampal glutamatergic neurotransmission and abnormal glucocorticoid signaling, demonstrated by decreased insulin and glucocorticoid receptors and increased GluA1 and GLUN1 subunits. All of these changes can be attributed to the observed prediabetes induced cognitive decline. Microglia surveys the CNS environment under normal conditions, and promptly responds to neural damage through proliferative, hypertrophic, morphological, and migratory changes (37,38). Under normal quiescent conditions, immune mechanisms are also activated by environmental/psychological stimuli and positively regulate the remodeling of neural circuits, promoting memory consolidation, hippocampal LTP and neurogenesis (39). The enriched environment is able to prevent and/or delay the development of memory deficits caused by diabetes in rats, possibly by attenuating harmful microglial activation, thus helping to ameliorate the cognitive comorbidities associated with disease. In addition, alterations in vasculature, oxidarive stress, lipid peroxidation, mitochondrial function, neuroinflammation, and other pathways have been implicated in diabetes-induced cognitive deficits.

Recalibrating life style is a prime therapeutic model to treat the clinical prediabetic condition. Several reports have shed light on the positive effects of an enriched environment on diabetes induced cognitive deficits, while also increasing hippocampal neurogenesis and dendritic extension (40). However, to the best of our knowledge, the effect of an enriched environment in the prediabetic condition has not yet been reported, which was addressed comprehensively in this study. Exposing the animals to different durations of an enriched environment for over 9 weeks did not influence the development of a prediabetes-like condition in the HSu fed Wistar rats. However, at longer exposure hours, the enriched environment certainly influenced the prediabetes induced cognitive changes in these study rats. The prediabetic rats exposed to 6 hours of the enriched environment (HSu+6h group) every day, scored significantly better in their anxiety levels and were more explorative in the assigned open field and

elevated plus maze tasks, compared to the prediabetic rats which received no such exposure. These rats also demonstrated a significantly better spatial learning and memory abilities too, as evidenced through the water maze test. Under the microscope, despite the rats being prediabetic, the hippocampal microstructure was found to be well retained in the HSu+6h group rats, as demonstrated by their significantly better dendritic density compared to the unexposed group. The prediabetic rats that were exposed to 4 hours of the enriched environment also performed better than the naive rats, with their scores reaching significance in a few of the assessments. All of this demonstrated a dose dependent effect of the enriched environment on the cognitive performance of the prediabetic rats. The ability of the enriched environment to increase neurogenesis, synaptic branching and dendritic spine generation in diabetic rats, may explain its positive effects in prediabetes induced cognitive and behavioral disorders, and impaired hippocampal plasticity (41-43). However, it is essential to unveil the underlying molecular mechanisms, the results of which should be helpful in designing the better-targeted therapies.

In summary, the results of this investigation confirmed our hypothesis. Prediabetes as an impaired metabolic condition inflict the cognitive/behavioral disorders in Wistar rats, as well as significantly reduces the hippocampal dendritic spine density, while providing these rats a long-term exposure to an enriched environment can overcame this phenomenon in a dose dependent manner. However, the underlying molecular mechanisms need to be unveiled which would warrant future studies.

6. ACKNOWLEDGMENTS

Feiyan Fan, Jing Qi contributed equally to this work. This work was supported by the National Natural Scientific Foundation of China for Jianfang Fu (No. 81670736), International science and technology cooperation and exchange program project of Shaanxi Province for Jianfang Fu (No. 2016KW-002), National Natural Scientific Foundation of China for Yanyang Tu (No. 81572983), The key research and development plan project of Shaanxi province for Yanyang Tu (No. 2017KW-062).

7. REFERENCES

1. Mijnhout GS, Scheltens P, Diamant M, Biessels GJ, Wessels AM, Simsek S, Snoek FJ, Heine RJ: Diabetic encephalopathy: A concept in need of a definition. *Diabetologia*, 49 (6), 1447-8 (2006)
DOI: 10.1007/s00125-006-0221-8
PMid:16598451

2. Schubert M, Brazil DP, Burks DJ, Kushner JA, Ye J, Flint CL, Farhang-Fallah J, Dikkes P, Warot XM, Rio C, Corfas G, White MF: Insulin receptor substrate-2 deficiency impairs brain growth and promotes tau phosphorylation. *J Neurosci*, 23 (18), 7084-92 (2003)
DOI:10.1523/JNEUROSCI.23-18-07084.2003
PMid:12904469

3. Wang JQ, Yin J, Song YF, Zhang L, Ren YX, Wang DG, Gao LP, Jing YH: Brain aging and AD-like pathology in streptozotocin-induced diabetic rats. *J Diabetes Res*. 2014, 796840 (2014)

4. Buysschaert M, Medina JL, Buysschaert B, Bergman M: Definitions (and Current Controversies) of Diabetes and Prediabetes. *Curr Diabetes Rev*, 12 (1), 8-13 (2016)
DOI: 10.2174/1573399811666150122150233
PMid:25612821

5. Tabák AG, Herder C, Rathmann W, Brunner EJ, Kivimäki M: Prediabetes: a high-risk state for diabetes development. *Lancet*, 379 (9833), 2279-90 (2012)
DOI: 10.1016/S0140-6736(12)60283-9

6. Yaffe K, Blackwell T, Kanaya AM, Davidowitz N, Barrett-Connor E, Krueger K: Diabetes, impaired fasting glucose, and development of cognitive impairment in older women. *Neurology*, 63 (4), 658-63 (2004)
DOI: 10.1212/01.WNL.0000134666.64593.BA
PMid:15326238

7. Biessels GJ, Strachan MW, Visseren FL, Kappelle LJ, Whitmer RA: Dementia and cognitive decline in type 2 diabetes and prediabetic stages: towards targeted interventions. *Lancet Diabetes Endocrinol,* 2 (3), 246-55 (2014)
DOI: 10.1016/S2213-8587(13)70088-3

8. Jones A, Kulozik P, Ostertag A, Herzig S: Common pathological processes and transcriptional pathways in Alzheimer's disease and type 2 diabetes. *J Alzheimers Dis*, 16 (4), 787-808 (2009)
DOI: 10.3233/JAD-2009-0973
PMid:19387113

9. Swardfager W, Lanctôt K, Rothenburg L, Wong A, Cappell J, Herrmann N: A meta-analysis of cytokines in Alzheimer's disease. *Biol Psychiatry,* 68 (10), 930-41 (2010)
DOI: 10.1016/j.biopsych.2010.06.012
PMid:20692646

10. Ramkumar K, Srikumar BN, Venkatasubramanian D, Siva R, Shankaranarayana Rao BS, Raju TR:

Reversal of stress-induced dendritic atrophy in the prefrontal cortex by intracranial self-stimulation. *J Neural Transm (Vienna)*, 119 (5), 533-43 (2012)
DOI: 10.1007/s00702-011-0740-4
PMid:22167578

11. den Heijer T, Vermeer SE, van Dijk EJ, Prins ND, Koudstaal PJ, Hofman A, Breteler MM: Type 2 diabetes and atrophy of medial temporal lobe structures on brain MRI. *Diabetologia*, 46 (12), 1604-10 (2003)
DOI: 10.1007/s00125-003-1235-0
PMid:14595538

12. S Roriz-Filho J, Sá-Roriz TM, Rosset I, Camozzato AL, Santos AC, Chaves ML, Moriguti JC, Roriz-Cruz M: (Pre)diabetes, brain aging, and cognition. Biochim Biophys *Acta*, 1792 (5), 432-43 (2009)
DOI: 10.1016/j.bbadis.2008.12.003
PMid:19135149

13. Arnold SE, Lucki I, Brookshire BR, Carlson GC, Browne CA, Kazi H, Bang S, Choi BR, Chen Y, McMullen MF, Kim SF: High fat diet produces brain insulin resistance, synaptodendritic abnormalities and altered behavior in mice. *Neurobiol Dis*, 67, 79-87 (2014)
DOI: 10.1016/j.nbd.2014.03.011
PMid:24686304 PMCid:PMC4083060

14. Faherty, C.J., D. Kerley, R.J. Smeyne: A Golgi-Cox morphological analysis of neuronal changes induced by environmental enrichment. *Brain Res Dev Brain Res*, 141 (1-2), 55-61 (2003)

15. Artola A, von Frijtag JC, Fermont PC, Gispen WH, Schrama LH, Kamal A, Spruijt BM: Long-lasting modulation of the induction of LTD and LTP in rat hippocampal CA1 by behavioural stress and environmental enrichment. *Eur J Neurosci*, 23 (1), 261-72 (2006)
DOI: 10.1111/j.1460-9568.2005.04552.x
PMid:16420435

16. Duffy SN, Craddock KJ, Abel T, Nguyen PV: Environmental enrichment modifies the PKA-dependence of hippocampal LTP and improves hippocampus-dependent memory. *Learn Mem*, 8 (1), 26-34 (2001)
DOI: 10.1101/lm.36301
PMid:11160761 PMCid:PMC311356

17. Segovia G, Del Arco A, De Blas M, Garrido P, Mora F: Environmental enrichment increases the *in vivo* extracellular concentration of dopamine in the nucleus accumbens: a microdialysis study. *J Neural Transm (Vienna)*, 117 (10), 1123-30 (2010)
DOI: 10.1007/s00702-010-0447-y
PMid:20706747

18. Hescham S, Jahanshahi A, Meriaux C, Lim LW, Blokland A, Temel Y: Behavioral effects of deep brain stimulation of different areas of the Papez circuit on memory- and anxiety-related functions. *Behav Brain Res,* 292, 353-60 (2015)
DOI: 10.1016/j.bbr.2015.06.032
PMid:26119240

19. Komada M, Takao K, Miyakawa T: Elevated plus maze for mice. *J Vis Exp,* (22)*,* 1088 (2008)

20. Montgomery, KC: The relation between fear induced by novel stimulation and exploratory behavior. *J Comp Physiol Psychol*, 48 (4), 254-60 (1955)
DOI: 10.1037/h0043788
PMid:13252152

21. Christine M. Walsh, Victoria Booth and Gina R. Poe: Spatial and reversal learning in the Morris water maze are largely resistant to six hours of REM sleep deprivation following training. *Learn Mem*, 18 (7), 422-434 (2011)
DOI: 10.1101/lm.2099011
PMid:21677190 PMCid:PMC3125613

22. Tan AM, Choi JS, Waxman SG, Hains BC: Dendritic spine remodeling after spinal cord injury alters neuronal signal processing. *J Neurophysiol*, 102 (4), 2396-409 (2009)
DOI: 10.1152/jn.00095.2009
PMid:19692517

23. Ramos-Rodriguez JJ, Ortiz O, Jimenez-Palomares M, Kay KR, Berrocoso E, Murillo-Carretero MI, Perdomo G, Spires-Jones T, Cozar-Castellano I, Lechuga-Sancho AM, Garcia-Alloza M: Differential central pathology and cognitive impairment in pre-diabetic and diabetic mice. *Psychoneuroendocrinology*, 38 (11), 2462-75 (2013)
DOI: 10.1016/j.psyneuen.2013.05.010
PMid:23790682

24. Reynolds RM, Strachan MW, Labad J, Lee AJ, Frier BM, Fowkes FG, Mitchell R, Seckl JR, Deary IJ, Walker BR, Price JF: Morning cortisol levels and cognitive abilities in people with type 2 diabetes: the Edinburgh type 2 diabetes study. *Diabetes care*, 33 (4), 714-20 (2010)
DOI: 10.2337/dc09-1796
PMid:20097784 PMCid:PMC2845011

25. Wang, S.B., J.P. Jia: Oxymatrine attenuates diabetes-associated cognitive deficits in rats. *Acta Pharmacol Sin*, 35 (3), 331-8 (2014) DOI: 10.1038/aps.2013.158 PMid:24442148 PMCid:PMC4647892

26. Buysschaert M, Medina J L, Bergman M, Shah A, Lonier J: Prediabetes and associated disorders. *Endocrine*, 48 (2), 1-23 (2014)

27. Tan ZS, Beiser AS, Fox CS, Au R, Himali JJ, Debette S, Decarli C, Vasan RS, Wolf PA, Seshadri S: Association of Metabolic Dysregulation With Volumetric Brain Magnetic Resonance Imaging and Cognitive Markers of Subclinical Brain Aging in Middle-Aged Adults. *Diabetes Care*, 34 (8), 1766-1770 (2011) DOI: 10.2337/dc11-0308 PMid:21680719 PMCid:PMC3142014

28. Ryan CM, Freed MI, Rood JA, Cobitz AR, Waterhouse BR, Strachan MW: Improving metabolic control leads to better working memory in adults with type 2 diabetes. *Diabetes care*, 29 (2), 345-51 (2006) DOI: 10.2337/diacare.29.02.06.dc05-1626 PMid:16443885

29. Burgeiro A, Cerqueira MG, Varela-Rodríguez BM, Nunes S, Neto P, Pereira FC, Reis F, Carvalho E: Glucose and Lipid Dysmetabolism in a Rat Model of Prediabetes Induced by a High-Sucrose Diet. *Nutrients*, 9 (6), (2017)

30. Soares E, Prediger R D, Nunes S, Castro AA, Viana SD, Lemos C, De Souza CM, Agostinho P, Cunha RA, Carvalho E, Fontes Ribeiro CA, Reis F, Pereira FC: Spatial memory impairments in a prediabetic rat model. *Neuroscience*, 250, 565 (2013)

31. Antunes LC, Elkfury JL, Jornada MN, Foletto KC, Bertoluci MC: Validation of HOMA-IR in a model of insulin-resistance induced by a high-fat diet in Wistar rats. *Arch Endocrinol Metab*, 60 (2), 138-42 (2016) DOI: 10.1590/2359-3997000000169 PMid:27191048

32. Reddy BR, Maitra S, Jhelum P, Kumar KP, Bagul PK, Kaur G, Banerjee SK, Kumar A, Chakravarty S: Sirtuin 1 and 7 mediate resveratrol-induced recovery from hyper-anxiety in high-fructose-fed prediabetic rats. *J Biosci*, 41 (3), 407-17 (2016) DOI: 10.1007/s12038-016-9627-8 PMid:27581932

33. Stranahan AM, Lee K, Martin B, Maudsley S, Golden E, Cutler RG, Mattson MP: Voluntary exercise and caloric restriction enhance hippocampal dendritic spine density and BDNF levels in diabetic mice. *Hippocampus*, 19 (10), 951-61 (2009) DOI: 10.1002/hipo.20577 PMid:19280661 PMCid:PMC2755651

34. Sale A, Maya Vetencourt JF, Medini P, Cenni MC, Baroncelli L, De Pasquale R, Maffei L: Environmental enrichment in adulthood promotes amblyopia recovery through a reduction of intracortical inhibition. *Nat Neurosci*, 10 (6), 679-681 (2007) DOI: 10.1038/nn1899 PMid:17468749

35. Cancedda L, Putignano E, Sale A, Viegi A, Berardi N, Maffei L: Acceleration of visual system development by environmental enrichment. *J Neurosci*, 24 (20), 4840-4848 (2004) DOI: 10.1523/JNEUROSCI.0845-04.2004 PMid:15152044

36. Landi S, Sale A, Berardi N, Viegi A, Maffei L, Cenni MC: Retinal functional development is sensitive to environmental enrichment, a role for BDNF. *FASEB J*, 21 (1), 130-139 (2007) DOI: 10.1096/fj.06-6083com PMid:17135370

37. Perry VH, Teeling J: Microglia and macrophages of the central nervous system, the contribution of microglia priming and systemic inflammation to chronic neurodegeneration. *Semin Immunopathol*, 35 (5), 601–612 (2013) DOI: 10.1007/s00281-013-0382-8 PMid:23732506 PMCid:PMC3742955

38. Grigsby JG, Cardona SM, Pouw CE, Muniz A, Mendiola AS, Tsin AT, Allen DM, Cardona AE. The role of microglia in diabetic retinopathy. *J Ophthalmol*, 2014, 705783 (2014)

39. Piazza FV, Segabinazi E, Centenaro LA, do Nascimento PS, Achaval M, Marcuzzo S: Enriched environment induces beneficial effects on memory deficits and microglial activation in the hippocampus of type 1 diabetic rats. *Metab Brain Dis*, 29 (1), 93–104 (2014) DOI: 10.1007/s11011-013-9467-2 PMid:24318482

40. Rahmeier FL, Zavalhia LS, Tortorelli LS, Huf F, Géa LP, Meurer RT, Machado AC, Gomez

R, Fernandes MDC: The effect of taurine and enriched environment on behaviour, memory and hippocampus of diabetic rats. *Neurosci Lett*, 630: 84-92 (2016)

41. Nithianantharajah J, Hannan AJ: Enriched environments, experience-dependent plasticity and disorders of the nervous system. *Nat Rev Neurosci*, 7 (9), 697-709 (2006)
DOI: 10.1038/nrn1970
PMid:16924259

42. Piazza FV, Pinto GV, Trott G, Marcuzzo S, Gomez R, Fernandes Mda C: Enriched environment prevents memory deficits in type 1 diabetic rats. *Behav Brain Res*, 217 (1), 16-20 (2011)
DOI: 10.1016/j.bbr.2010.09.017
PMid:20888365

43. Beauquis J, Roig P, De Nicola AF, Saravia F: Short-term environmental enrichment enhances adult neurogenesis, vascular network and dendritic complexity in the hippocampus of type 1 diabetic mice. *PloS one*, 5 (11), e13993 (2010)
DOI: 10.1371/journal.pone.0013993
PMid:21085588 PMCid:PMC2981567

Abbreviations: impaired fasting glucose (IFG), impaired glucose tolerance (IGT), Tyep 2 Diabetes Mellitus (T2DM), enriched environment (EE), dentate gyrus (DG), high sucrose (HSu), fasting blood sugar (FBS), fasting serum insulin (FINs), elevated plus maze (EPM).

Key Words: Prediabetes, Cognitive Dysfunction, Hippocampus, Enriched Environment

Send correspondence to: Yanyang Tu, Department of Experimental Surgery, Tangdu Hospital, The Fourth Military Medical University, Xi'an, 710038, Shanxi, China, Tel: 029-84778169, Fax: 029-84775217, E-mail: ayonst@163.com