Probiotics treatment improves diabetes-induced Impairment of synaptic activity and cognitive function: behavioral and...
PROBIOTICS TREATMENT IMPROVES DIABETES-INDUCED IMPAIRMENT OF SYNAPTIC ACTIVITY AND COGNITIVE FUNCTION: BEHAVIORAL AND ELECTROPHYSIOLOGICAL PROOFS FOR MICROBIOME–GUT–BRAIN AXIS

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Abstract—Diabetes mellitus-induced metabolic disturbances underlie the action of many systems including some higher functions of the brain such as learning and memory. Plenty of evidence supports the effects of probiotics on the function of many systems including the nervous system. Here we report the effect of probiotics treatment on the behavioral and electrophysiological aspects of learning and memory disorders. Diabetic rats were made through intraperitoneal injection of streptozocin. The control and diabetic rats were fed with either normal regimen (control rats receiving normal regimen (CO) and diabetics rats receiving normal regimen (DC), respectively) or normal regimen plus probiotic supplementation for 2 months (control rats receiving probiotic supplementation (CP) and diabetics rats receiving probiotic supplementation (DP), respectively). The animals were first introduced to spatial learning task in the Morris water maze. Then, in electrophysiological experiments, stimulating the Schaffer collaterals the basic and potentiated excitatory postsynaptic potential (EPSPs) were recorded in the CA1 area of the hippocampus. Finally, the serum levels of glucose, insulin, superoxide dismutase and 8-hydroxy-2-deoxyguanosine (8-OHGd) were measured. We found that probiotics administration considerably improved the impaired spatial memory in the diabetic animals. The probiotics supplementation in the diabetic rats recovered the declined basic synaptic transmission and further restored the hippocampal long-term potentiation (LTP). While the probiotics administration enhanced the activation of superoxide dismutase and increased the insulin level of serum it decreased both the glucose level of serum and the 8-OHGd factor. From the present results we concluded that probiotics efficiently reverse deteriorated brain functions in the levels of cognitive performances and their proposed synaptic mechanisms in diabetes mellitus. These considerations imply on the necessity of an optimal function of the microbiome–gut–brain axis in the behavioral as well as electrophysiological aspects of brain action.

Key words: diabetes, LTP, oxidative stress, probiotics, spatial memory.

INTRODUCTION

Diabetes mellitus is a disease caused by absolute or relative insulin deficiency (Vicentini et al., 2011). It is well documented that oxidative stress is a basic mechanism behind the development of the diabetic state (Aly and Mantawy, 2012). Hyperglycemia in diabetes mellitus (DM) may be one of the most important factors responsible for the development of oxidative stress, which promotes the main complications in DM patients (Vicentini et al., 2011). An imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage, is termed ‘oxidative stress’ (Sies, 1997). Oxidative stress and the damage that it causes have been implicated in a wide variety of natural and pathological processes, including diabetes, aging, cancer, atherosclerosis, neurological degeneration, schizophrenia, and autoimmune disorders such as arthritis (Shackelford et al., 2000). Oxidative stress can be derived from a variety of sources that include events such as the production of reactive oxygen species by mitochondrial oxidative phosphorylation, ionizing radiation exposure, and metabolism of exogenous compounds (Shackelford et al., 2000). In addition to these sources of oxidative stress, a decrease in the activity of antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase, and catalase may contribute to some disease states (Sagara et al., 1998).

Several biomarkers to estimate oxidative stress have been suggested, however, most of them have failed to reach clinical significance. One successful discovery of the late 1980s was the level of 8-hydroxydeoxyguanosine (8-hydroxy-2-deoxyguanosine; 8-OHGd) which has been proven to be increased in the serum or urine of patients who have oxidative stress-associated disease (Lunec et al., 2002). Reactive oxygen species attacks guanine bases in DNA easily and form 8-OHGd which can bind to...
The probiotics administration on the induction of LTP? How do behavioral tasks a food regimen containing probiotics affect the serum level of genotypic and phenotypic options that would otherwise be inaccessible (Deeds et al., 2011).

Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (Guarner et al., 2005). Widely accepted probiotics contain different lactic acid producing bacteria of human origin including bifidobacteria, lactobacilli or enterococci (Songiessp et al., 2005). In order to improve the efficacy of probiotics, combinations of different bacterial strains can be used (Karimi and Pena, 2003). A common choice is a mixture of Lactobacillus and Bifidobacteria (Karimi and Pena, 2003). Probiotics are used in the treatment of a range of diseases such as infections, allergies, and inflammatory disorders (Isolauri and Salminen, 2008).

Through decreased inflammatory damages and increased level of antioxidant enzymes such as SOD and glutathione peroxidase probiotics suppress the production of the oxidative stresses (D’Souza et al., 2010). STZ-induced DM offers a very cost-effective and expeditious technique that can be used in most strains of rodents, opening the field of DM research to an array of genotypic and phenotypic options that would otherwise be inaccessible (Deeds et al., 2011).

In the present study we asked several questions: Does a food regimen containing probiotics affect the serum level of glucose and insulin? How do the behavioral tasks respond to the probiotics treatment? What is the effect of the probiotics administration on the induction of LTP?

**EXPERIMENTAL PROCEDURES**

**Experimental design**

The effect of probiotics on spatial learning and memory, hippocampal LTP and some oxidative stress biomarkers was studied in rats. After 8 weeks of probiotics administration, the animals were tested in a Morris water maze. Then, electrophysiological recordings were carried out in the CA3–CA1 pathway of the hippocampus. Eventually, some oxidative stress biomarkers in the serum were measured when the electrophysiological experiments were finished.

**Animals and feeding schedule**

Forty male Wistar rats at 45 days of age were used in this study. The animals were randomly assigned to four groups (n = 10 in each group, one animal per cage). control rats receiving normal regimen (CO) and diabetic rats receiving normal regimen (DC) were fed with standard Chow. Also control rats receiving probiotic supplementation (CP) and diabetic rats receiving probiotic supplementation (DP) rats were administered with probiotics (1 g/rat/12 h) in drinking water. The animals were kept under an air-conditioned (humidity 50–60%) and temperature-controlled (22 °C) room with 12-h light/12-h dark cycle beginning at 6:00 AM.

**Preparation of the probiotics solution**

The probiotics solution was made by a mixture (each 334 mg) of Lactobacillus acidophilus (American type culture collection (ATCC) 4356, ~10^10 cfu/g), Bifidobacterium lactis (Dutch chemical company (DSM) 10140, ~10^10 cfu/g) and Lactobacillus fermentum (ATCC 9338, ~10^10 cfu/g) dissolved in drinking water. The CP and DP (starting the next day after the onset of diabetes) groups received the probiotics twice daily for 56 days before beginning the experiments; the treatment continued through electrophysiological experiments. The probiotics were obtained from 2ist takhmir company, Tehran, Iran. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the Isfahan University of Medical Sciences.

**Diabetic model of rats**

Diabetic rats were made by a single intraperitoneal injection of STZ at 65 mg/kg (Sigma–Aldrich, USA) dissolved in 0.1 M citrate buffer (pH = 4.5). The animals were considered diabetic with blood glucose levels >200 mg/dl 5 days after the STZ injection. The fasting blood glucose was measured at the end of experiments.

**Behavioral experiments**

All experiments were performed in a quiet and darkened room illuminated only by sparse light. Each group was equally represented at the times of testing. For adaptation the animals were placed into the room for two hours prior to the experiment.

Spatial representations were assessed in a Morris water maze as described previously (Taghizadeh et al., 2011). A hidden platform was placed on a fixed location in the center of one of four supposed quadrants of the pool. During the acquisition (learning) phase the animals were trained to locate the platform. A trial was begun by randomly placing the rat in water facing the wall of the pool at one of the four starting points. The animals were allowed to swim freely until they found the platform. Each rat was given 60 s to find the hidden platform and was allowed to stay on the platform for 15 s. On 3 consecutive days the animals were given four acquisition trials each day and the latency time swum to reach the platform was measured. Then the animals were introduced to a retrieval test after finishing the fourth trial on the final day while the platform was removed. The percentage of quadrant time (the time spent by the rat in target quadrant where the platform was formerly placed) was recorded and calculated.

**Electrophysiological experiments**

**Surgery and recordings.** Surgical procedures and electrophysiological recordings were performed as described previously (Talaee et al., 2010). The experimental subjects were anesthetized by urethane (1.5 g/kg, i.p.) and placed in a stereotaxic frame. Two holes were drilled in the skull: a hole for the stimulating electrode (1 mm diameter, 4.2 mm posterior to bregma, 3.8 mm lateral to the midline) and another one for the recording electrode (1 mm diameter, 3.4 mm posterior to...
bregma, 2.5 mm lateral to the midline). The recording and stimulating electrodes were lowered into the CA1 stratum radiatum and the Schaffer collaterals, respectively. In response to test pulses (two sweeps/min at 30-s intervals) applied to the Schaffer collaterals evoked excitatory postsynaptic potential (EPSPs) of the CA1 field were attained. The stimulation intensity was adjusted to a level that evoked 60% of maximum EPSP amplitude. The EPSPs were recorded at 30-s intervals for 30-min period and the baseline data were obtained by averaging the response. Then, LTP was induced by high-frequency stimulation (HFS) of 100 Hz (10 bursts of 10 stimuli, 0.1-ms stimulus duration, and 2-s inter-burst intervals). Following the tetanus, responses to the test pulses were collected continuously for at least 2 h. The data were considered for the pre- and post-tetanic changes of response. The size of the amplitude was measured from the baseline to the lowest point of the EPSP.

Biochemical measurements. Blood samples were prepared from all animals when the electrophysiological experiments were finished. The samples were kept at 4°C for 30 min for clotting. Clear serum was obtained by centrifugation of the clotted blood samples at 2500 rpm for 5 min and was kept frozen until used for measurements. Serum concentration of glucose was measured using a glucometer. The serum levels of insulin, oxidative stress biomarkers SOD and 8-OHdG were measured using a rat enzyme-linked immunosorbent assay (ELISA) kit (purchased from Cayman, USA).

Statistical analysis

The data taken from acquisition phase of the behavioral testing were analyzed by Two-way repeated measures analysis of variance (ANOVA). Two-way ANOVA was applied to the probe tests and the biochemical assessments as well. Three-way ANOVA was used to assess the variations between the baseline and post-tetanus recordings. LTP was expressed as mean percent change of amplitude in the post-HFS recordings over 120 min in comparison to the baseline amplitude. All statistics were followed by the Bonferroni post hoc test if significant. The probability levels were interpreted as statistically significant if the $P$ value was less than 0.05.

RESULTS

Spatial learning and memory

Acquisition phase. Navigation of the animals in the Morris water maze was evaluated as spatial learning and memory. The animals were tested at four trials/day for 3 days and the data of each day were averaged as one point (Fig. 1). Analysis of variance confirmed a statistical variation between function of the four groups of animals in the water maze ($F_{3, 156} = 37.623; P < 0.0001$). Whereas CO rats displayed a high performance in locating the hidden platform the DC animals showed the least capability in learning the task. Statistics indicated superiority of the CO group on the DC one in maze navigation ($P < 0.0001$). Treatment with the probiotics markedly influenced the maze searching in either control or diabetic rats. The DP animals improved their spatial performance so that they found the hidden platform within a shorter time in the correct quadrant than did the DC animals ($P = 0.008$). Interestingly, the probiotics administration could close the maze searching of the DP rats to the CO ones ($P = 0.373$). The CP subjects further improved their function in the water maze displaying the highest performance compared to the other groups ($P < 0.0001$). Fig. 1 depicts the capability of the testing animal groups in learning the spatial task.

Probe trial phase. The animals were released in the water maze while the platform was removed and they were allowed to swim freely for 30 s. Analysis of variance indicated that the differences between locating the target quadrant by the four testing groups were statistically significant ($F_{3, 36} = 9.069; P < 0.0001$). The vehicle-treated CO and DC rats spent $12.20 \pm 0.97$ s and $6.11 \pm 0.67$ s in the correct quadrant, respectively. The function of the two groups was varied significantly ($P < 0.0001$). The probiotic-treated DP animals prominently improved their performance so that they remained in the target quadrant $10.18 \pm 0.94$ s, significantly higher than the DC rats ($P = 0.01$) and almost near to the CO group ($P = 0.613$). On the other hand, the regimen consisting of probiotics was not effectively influenced the behavior of the CP group in the water maze ($10.24 \pm 0.79; P = 0.673$, the CO vs. CP animals). The behavior of animals in the retrieval tests is illustrated in Fig. 2.

Electrophysiological recordings. In the electrophysiological experiments the effect of probiotics
on the pre- and post-tetanus EPSPs in the hippocampus of the control and diabetic animals was evaluated. Analysis of variance indicated a general significant difference between pre- and post-HFS recordings in the four testing groups ($F_{7, 1592} = 407.733; P < 0.0001$).

**Baseline responses.** Mean amplitude of the evoked EPSPs recorded in the CA1 area of hippocampus of the CO rats was $0.94 \pm 0.03$ mV. It markedly decreased to $0.42 \pm 0.02$ mV in the DC rats ($P < 0.0001$, the CO vs. DC rats). Probiotics administration yielded a considerable increase in the amplitude of the baseline responses in the diabetic rats ($0.87 \pm 0.03$; $P < 0.0001$, the DP vs. DC rats) but a negligible change in their CP counterparts ($0.97 \pm 0.02$). ANOVA indicated no difference between characteristics of the basic EPSPs in the CP and CO animals (Fig. 3).

**Induction of LTP.** The Schaffer collateral-CA1 pathway was tetanized for the induction of LTP in the EPSPs recorded in the CA1 area. The HFS elicited an abrupt LTP in the CO animals ($58.3 \pm 2.49\%$, $P < 0.0001$) and the potentiation persisted as long as the recording continued. The tetanic stimulation induced an initial transient enhancement in the DC rats. It showed a sharp decay so that the recordings appeared even a slight depression at the end of experiments. ANOVA indicated a significant variation between the CO and DC groups ($P < 0.0001$). Probiotic administration restored LTP in the DP animals ($34.04 \pm 2.92\%$; $P < 0.0001$) with a considerable variation compared to the DC rats ($P < 0.0001$). However, the post-tetanus enhancement turned to the baseline when the recordings finished. Despite the occurrence of this substantial LTP in the DP animals the difference observed between this group and the CO group was statistically noticeable ($P < 0.0001$).

In the CP group, on the other hand, administration of the probiotics led to an insignificant decrease in the magnitude of post-HFS potentiation ($40.83 \pm 1.49\%$; $P = 0.071$, the CO vs. CP animals). Fig. 4 compares the trend of post-HFS changes in the vehicle and probiotic-treated control and diabetic animals.

**Biochemical assessments.** After 2 months of probiotic treatment the fasting blood glucose, the level of serum insulin and the stress oxidative biomarkers were measured.

**Serum glucose concentration.** Induction of the diabetes expectedly led to a vigorous rise of serum concentration of glucose. The mean value of serum glucose in the DC rats was $520.60 \pm 28.15$ mg/dl, more than five times compared to their CO counterparts ($96.8 \pm 3.3$ mg/dl, $P < 0.0001$). The DP animals
Fig. 4. Induction of LTP in the EPSPs recorded in the CA1 area after tetanization of the CA3–CA1 pathway. Although tetanus elicited the greatest LTP in the CO rats it failed to potentiate responses in the DC group. While probiotics treatment considerably triggered LTP in the DP group it caused a decreased post-tetanus potentiation in the CP animals, although the difference between the CO and CP rats is not significant. Statistics indicated significant differences between the CO rats with the DC and DP groups (P < 0.0001), and, between the DC and DP groups. Each point represents an average of 3-min recordings. HFS indicates the time of application of the tetanic stimulation.

Fig. 5. (A) Plasma glucose concentration (mg/dl) was measured after finishing the electrophysiological experiments. The level of serum glucose was prominently increased in the STZ-injected DC animals. Although probiotics administration markedly declined the blood sugar in the DP group it had no important effect on the plasma glucose of the CP rats. *P < 0.0001 DC vs. CO, #P = 0.006 DP vs. DC, *P < 0.0001 CO vs. DP. (B) The insulin level of plasma (ng/ml) was measured in the four testing groups when the electrophysiological experiments were finished. The vehicle-treated DC rats showed the least content of serum insulin. The regimen supplemented with probiotics efficiently increased the insulin of plasma in the DP group but left that in the CP animals unchanged. *P < 0.0001 DC vs. CO and DC vs. DP. The values are presented as mean ± SEM.
receiving the probiotics had significantly lesser serum glucose (410.10 ± 33.44 mg/dl) compared to the DC group (P = 0.006) although it was still higher than in the CO rats (P < 0.0001). The serum level of glucose was unchanged in the probiotic-treated CP animals (101.6 ± 2.87 mg/dl). Fig. 5A summarizes the effect of probiotics supplement on the control and diabetic rats.

Serum insulin concentration. The insulin level of blood in the CO group was 7.25 ± 0.22 ng/ml. Injection of STZ to the DC animals decreased the serum insulin to 3.26 ± 0.14 ng/ml, to almost half compared to the CO rats (P < 0.0001). The probiotic treatment effectively enhanced the insulin production to a mean value of 5.88 ± 0.19 ng/ml in the DP animals (P < 0.0001, the DC vs. DP group). The probiotic supplementation did not influence the insulin level of serum in the CP rats. Fig. 5B depicts how probiotics influence insulin concentration of serum.

The effect of probiotics on the stress oxidative factors

Superoxide desmutase enzyme. Induced diabetes in the DC animals considerably reduced activity of the SOD enzyme when compared to the CO rats (P < 0.0001, the DC vs. CO rats). Although probiotics administration significantly increased activity of the enzyme in the DP group (P = 0.007, the DC vs. DP rats), however, the difference was still evident between the CO and DP groups (P < 0.034). Probiotics negligibly changed the activity of the SOD enzyme in the CP animals. Fig. 6A demonstrates the efficacy of the probiotics on activity level of the SOD enzyme.

8-OHdG production. The biomarker 8-OHdG is produced as a result of DNA degradation. The 8-OHdG was highly increased in the DC group (P < 0.0001, the DC vs. CO rats). Receiving a regimen supplemented with probiotics significantly inhibited production of the biomarker (P < 0.0001, the DC vs. DP animals), however, its amount was still high compared to the CO group (P = 0.001). Probiotics administration could not effectively influence production of the 8-OHdG in the CP rats. Fig. 6B illustrates how the probiotic treatment affects the production of 8-OHdG factor.

DISCUSSION

Experiments on the control animals

The metabolic disease diabetes mellitus, especially in its advanced form, profoundly affects the function of the nervous system (Selvarajah et al., 2011). This study evaluates the behavioral and electrophysiological aspects of brain functions. In the behavioral experiments

![Fig. 6. (A) Histograms show the activity level (U/mL) of superoxide dismutase enzyme (SOD) in the vehicle and probiotic-treated control and diabetic groups. Diabetes significantly decreased the activity of the enzyme in the DC group. The probiotics treatment effectively elevated the enzyme activity in only the DP rats. *P < 0.0001 DC vs. CO, **P = 0.007 DP vs. DC, #P = 0.034 CO vs. DP. (B) Illustration of changes in the DNA degradation factor (8-OHdG) in the vehicle and probiotic-treated control and diabetic rats. Degradation of the factor was prominently enhanced in the DC rats. Probiotics supplementation significantly prevented the factor degradation in the DP, but not CP, animals. *P = 0.001 DP vs. CO, **P < 0.0001 DC vs. CO and DP. The values are presented as mean ± SEM.](image-url)
we found that the STZ-injected rats were reasonably inferior to their control counterparts in performing the spatial tasks. Learning and memory are higher functions of the brain that are effectively influenced by diabetes (Jiang et al., 2012). Neurobehavioral researches indicate learning deficits in subjects with diabetes (Biessels and Gispen, 2005; Alvarez et al., 2009). It has been shown that diabetic rats failed on some behavioral tasks, such as passive avoidance (Grzeda et al., 2007), object learning and radial maze (Kamal et al., 2000). Moderate disorders of learning and memory have also been reported in both type 1 and 2 diabetic patients (Biessels and Gispen, 2005). Additionally, evidence supports an association between diabetes mellitus, dementia and Alzheimer’s disease (Belanger et al., 2004).

Our in vivo electrophysiological experiments resulted in the same consequences as the behavioral findings. Stimulation of the CA3–CA1 pathway triggered declined basic EPSPs in the diabetic rats. Studies have indicated that some neurophysiological parameters are impaired in the diabetic rats. It is demonstrated that slowing of conduction velocity in diabetes is first measurable in the peripheral nervous system (2–4 weeks after the induction of diabetes) and subsequently in the central pathways (Biessels et al., 1999). Reports also specify that diabetes gives rise to promoting reductions and preventing enhancements of synaptic transmission (Ungless et al., 2001). Consistently, there are results showing reduced AMPA receptor binding (Chabot et al., 1997) as well as alterations of N-methyl-d-aspartic acid (NMDA) receptors (Wisniewski et al., 2003) in diabetic rats.

We considered LTP as an index for evaluating plasticity of synaptic transmission in the hippocampal neuronal circuit. In our experiments the tetanic stimulation of the Schaffer collaterals was rationally failed to induce the NMDA receptor-mediated LTP in the CA1 area of the hippocampus of diabetic animals. Electrically induced synaptic plasticity LTP has been known as a candidate mechanism for learning and memory. Studies support a declined occurrence of NMDA-dependent LTP in the CA1 field in diabetic subjects; it gradually appears and reaches a maximum of 12 weeks after the induction of diabetes (Biessels et al., 1996; Chabot et al., 1997; Kamal et al., 1999). It is reported that NMDA receptor expression and phosphorylation is down-regulated in postsynaptic densities from the brains of chronic streptozotocin-diabetic rats (Kamal et al., 1999). It is also suggested that the loss of LTP maintenance in diabetic animals results from the disruption of calcium-dependent processes that modulate postsynaptic AMPA receptors during synaptic potentiation (Chabot et al., 1997).

Interestingly, the extent of LTP prevention matches the severity of hyperglycemia (Gispen and Biessels, 2000) so as to moderate hyperglycemia (blood glucose approximately 15 mmol/l), there is no deficit in the induction of LTP (Tekkoot and Krnjevic, 1999).

The learning deficits in streptozotocin-induced diabetic rats are shown to be in line with changes in the hippocampal synaptic plasticity (Popovic et al., 2001).

Kamal et al. demonstrated that the disturbed hippocampal LTP and LTD in diabetic rats correlated well with the learning impairments (Kamal et al., 2000). Consistently, in our study the electrophysiological disorders in synaptic transmission match the behavioral deficits.

In the biochemical assessment the values closely match those taken from the spatial learning and memory as well as synaptic plasticity evaluations. In biochemical assessments we found that, as is expected, while the insulin level of serum was markedly decreased the glucose concentration of serum was noticeably elevated in the diabetic rats. It is well known that all forms of diabetes are characterized by hyperglycemia, whether relative or absolute lack of insulin action. Indeed, hyperglycemia is clearly recognized as the primary reason for the pathogenesis of diabetic complications (Evans et al., 2003).

The biochemical measurements also revealed a significant decrease in the activity of SOD enzyme and a marked increase in the DNA degradation factor 8-OHdG. Various mechanisms have been suggested to contribute to the formation of reactive free radicals (Evans et al., 2003). Diabetes is usually accompanied by increased production of free radicals (Shackelford et al., 2000) or impaired antioxidant defenses (Vicentini et al., 2011). Free radicals are formed disproportionately in diabetes by glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins (Maritim et al., 2003). Actually increased oxidative stress is a widely accepted participant in the development and progression of diabetes and its complications (Baynes and Thorpe, 1999). There are data indicating that reactive oxygen species (ROS) formation is a direct consequence of hyperglycemia (Brownlee, 2001). Since β-Cells are low in free-radical reducing enzymes such as catalase, glutathione peroxidase, and SOD they are sensitive to ROS (Evans et al., 2003) and reactive nitrogen species (RNS) (Tiedge et al., 1997). On the other hand, the activation of the stress oxidative factors is affected in the STZ-injected animals (Aly and Mantawy, 2012). However, the effect of diabetes on the activity of SOD is erratic, with no noticeable pattern based on gender or species of animal, or duration of diabetes, or tissue studied (Maritim et al., 2003).

Effects of probiotics supplementation

The probiotics treatment outstandingly reversed the behavioral and electrophysiological deficits. Probiotics positively affected learning of the maze tasks. The probiotic-treated diabetic rats highly improved performing both the acquisition and probe trial tests so that their behavioral function was close to the CO rats.

The regimen-containing probiotics had a pronounced effect on the basic synaptic transmission which is reflected in higher amplitude of the baseline responses. Also, the tetanic stimulation triggered a considerable LTP in the CA1 EPSPs of recorded in the DP animals. Probiotics administration also improved the biochemical indices. While the SOD activity was
increased the production of the 8-OHdG factor, reflecting degradation of DNA, was inhibited. The insulin level of serum was increased and, concomitantly, the serum concentration of glucose was diminished.

Interestingly, the probiotics administration affected neither the behavioral functions nor the placticity of synaptic transmission in the control rats. The same results were also observed in the biochemical measurements where the probiotics-supplemented regimen failed to underlie the level of serum insulin and glucose as well as the activity of the stress oxidative factors. Hence, from the present findings we concluded that under optimal activity of natural probiotics in the body, probiotics supplementation does not have a supportive role in brain function.

Do probiotics underlie the typical diabetes complications? It is shown that, through increased free radicals, diabetes mellitus reduces the antioxidant capacity of tissues (Sagara et al., 1998). While diabetes considerably decreases the levels of serum SOD (Arif et al., 2010) food regimens supplemented with prebiotics, probiotics, and symbiotics up-regulate SOD-1 (D'Souza et al., 2010). Probiotics inhibit oxidative stress via reducing inflammation and increasing antioxidant enzymes such as SOD and glutathione peroxidase (D’Souza et al., 2010). Researchers have indicated that probiotics may increase the antioxidant capacity of plasma, liver and intestines of animals, and decrease the malondialdehyde content in plasma (Uskova and Kravchenko, 2009). Yadav et al showed that probiotics administration suppress STZ-induced oxidative damage in pancreatic tissues by inhibiting the lipid peroxidation and formation of nitric oxide, and preventing antioxidant pool such as glutathione content and activities of SOD, catalase and glutathione peroxidase. They concluded that the suppression of STZ-induced consequences in carbohydrates and lipid metabolism as well as oxidative stress may be attributed to higher availability of biologically active substances in probiotic-treated diabetic animals (Yadav et al., 2008).

Probiotics inhibit the depletion of insulin as well as preserving diabetic dyslipidemia, and inhibit lipid peroxidation and nitrite formation, and thus, suppress STZ-induced diabetes in rats. This may enable the antioxidant system of β-Cells and may decelerate the reduction of insulin and the elevation of blood glucose levels (Yadav et al., 2008). Further, due to the protection of pancreatic β-Cells from damage, probiotics may delay the STZ-induced alterations in glucose homeostasis by maintaining insulin levels (Yadav et al., 2008).

The main part of our study was devoted to the evaluation of spatial performance in the STZ-induced diabetic rats, and LTP as a candidate mechanism of learning and memory. Classically the brain is viewed as an organ metabolizing glucose independent of insulin, however, growing biochemical evidence indicates expression of the insulin-sensitive glucose transporter glucose transporter-4 (GLUT-4) in CNS (Sankar et al., 2002). Findings confirm that insulin exerts pleiotropic effects in neurons, including the regulation of neuronal proliferation, apoptosis, synaptic transmission, neuronal degeneration, and learning (Schubert et al., 2004). In the behavioral assessment we found that the STZ-injected animals had a poor function in both learning and retention of the spatial task. The probiotics treatment led the diabetic rats to perform almost similar to the normal controls.

Attempts have been carried out to clarify relations between diabetes, CNS function and probiotics. A growing body of evidence suggests an interaction between intestinal microbiota, gut, and CNS in what is recognized as the microbiome–gut–brain axis (Cryan and O'Mahony, 2011). Fukui et al. reported that oxidative stress-induced damage to synapses in cerebral cortex and hippocampus during aging may results in deficit of cognitive function (Fukui et al., 2001). Zis et al. believe that antioxidant enzymes could be a potential target for the prevention of memory deterioration in adults with Down’s syndrome (Zis et al., 2012). Antioxidants might be of general use in the prevention of the neurodegeneration and cognitive functions associated with diabetes. For instance, vitamin E may act as an antioxidant to reduce oxidative damage to the synapses in the hippocampus (Fukui et al., 2001). It is reported that treatment with this vitamin prevented the learning and memory deficits induced by streptozotocin-diabetes. Clausen and his colleagues found that antioxidants and low doses of SOD and catalase protect cognitive functions from damages caused by stress oxidative factors (Clausen et al., 2010). From these considerations it can be concluded that through rising antioxidant enzymes, and in turn protection of the brain from cell damage, probiotics may prevent memory impairments in the STZ-induced diabetes.

Our results revealed that STZ-induced diabetes yields reduced basic synaptic activity as well as LTP in the CA3–CA1 pathway. The probiotics supplementation restored the synaptic transmission and LTP to normal values. So far, no information is evident about effect of probiotics on the occurrence of hippocampal LTP in the diabetic animal models. Researches indicate that the probiotics help to maintain insulin levels (Yadav et al., 2008) and effects of this hormone on neuronal function (Freychet, 2000). Such an effect is supportive to normal synaptic transmission in the neural circuits.

Two main excitatory glutamate receptors in the CA1 area of hippocampus are AMPA and NMDA receptors. Activation of both receptors and flowing positive ions through their ion channels is necessary for the induction of LTP. Concurrent occurrence of inhibitory postsynaptic potential (IPSPs) naturally reduces the probability of LTP induction. GABA, as a main inhibitory neurotransmitter in the brain, is involved in regulating numerous physiological and psychological processes. There is evidence indicating that the inactivation of hippocampal GABA_A receptors improves spatial working memory (Helm et al., 2005). In one study Bravo et al. showed that hippocampal GABA_A mRNA is reduced in probiotic-treated mice, which is consistent with an enhanced memory consolidation in a fear-conditioning
test in their study. They also found that the probiotics administration affected the transcripts of GABA<sub>Α</sub> receptor subunits in the hippocampus (Bravo et al., 2011). Human studies also provide evidence that intestinal microflora and probiotics play a role in central nervous system functions. It is reported that oral probiotics as well as gut microbial population have beneficial effects on mood and psychological distress such as decreased anxiety and depression; indicating a relationship between probiotics and stress-related psychopathologies (Bravo et al., 2011). How do the probiotics positively shift the thresholds toward the induction of synaptic plasticity? If probiotics restore diabetes-associated diminished LTP through a direct effect on the reduced activity of NMDA and AMPA receptors (Wisniewski et al., 2003), indirectly via the prevention of hyperglycemia; a potent status for the inhibition of LTP (Gispen and Biessels, 2000), changing the decelerated conduction velocity (Collison et al., 2012), and other possibilities need future investigations.

Overall, the present study demonstrates that treatment with probiotics pronouncedly improves performance of the diabetic animals in the spatial learning tasks. Also the diabetic animals receiving regimen-containing probiotics show an enhanced capability in the baseline synaptic activity as well as synaptic plasticity. Our findings from the behavioral and electrophysiological experiments open a window for concomitant evaluation of the learning and memory and, hippocampal synaptic plasticity in diabetes and how the probiotics generate similar positive effects on both phenomena. Additionally, these data imply on the necessity of an optimal function of the microbiome–gut–brain axis in the behavioral as well as electrophysiological aspects of brain action.

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