Effect of Environmental Enrichment Exposure on Neuronal Morphology of Streptozotocin-induced Diabetic and Stressed Rat Hippocampus

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Background: Environmental enrichment (EE) exposure is known to influence the structural changes in the neuronal network of hippocampus. In the present study, we evaluated the effects of EE exposure on the streptozotocin (STZ)-induced diabetic and stressed rat hippocampus.

Methods: Male albino rats of Wistar strain (4-5 weeks old) were grouped into normal control (NC), vehicle control (VC), diabetes (DI), diabetes + stress (DI + S), diabetes + EE (DI + E), and diabetes + stress + EE (DI + S + E) groups ($n = 8$ in each group). Rats were exposed to stress and EE after inducing diabetes with STZ (40 mg/kg). Rats were sacrificed on Day 30 and brain sections were processed for cresyl violet staining to quantify the number of surviving neurons in the CA1, CA3, and dentate hilus (DH) regions of hippocampus.

Results: A significant ($p < 0.001$) decrease in the number of survived neurons was noticed in DI (CA1, 34.06 ± 3.2; CA3, 36.1 ± 3.62; DH, 9.83 ± 2.02) as well as DI + S (CA1, 14.03 ± 3.12; CA3, 20.27 ± 4.09; DH, 6.4 ± 1.21) group rats compared to NC rats (CA1, 53.64 ± 2.96; CA3, 62.1 ± 3.34; DH, 21.11 ± 1.03). A significant ($p < 0.001$) increase in the number of survived neurons was observed in DI + E (CA1, 42.3 ± 3.66; CA3, 46.73 ± 4.74; DH, 17.03 ± 2.19) and DI + S + E (CA1, 29.69 ± 4.47; CA3, 36.73 ± 3.89; DH, 12.23 ± 2.36) group rats compared to DI and DI + S groups, respectively.

Conclusions: EE exposure significantly reduced the amount of neuronal damage caused by complications of diabetes and stress to the neurons of hippocampus.

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At a Glance Commentary

Scientific background of the subject

The neurological consequences of diabetes mellitus in the central nervous system have gained attention in recent times. Previous findings of the research discovered neuronal alterations and reduced neuronal proliferation in the diabetic hippocampus. Exposure to stress causes severe neuronal damage in the hippocampus. In this study we aimed to observe the neuroprotective effect of environmental enrichment exposure in the diabetic and stressed rat hippocampus.

What this study adds to the field

Outcomes of this study may be helpful in correlating and in understanding the complications of the diabetes and stress on the hippocampal neurons of brain. Methods used in this study may be helpful in the prevention of neuronal damage in the brain with the help of environmental enrichment exposure treatment. The data obtained in the study may be useful to interpret the functional deficits of diabetic patients and for the better management of diabetes.

Key words: diabetes, enrichment, hippocampus, neurons, streptozotocin, stress

Diabetes mellitus is an endocrine metabolic disorder and can lead to peripheral neuropathies which include motor and sensory polyneuropathies, as well as autonomic neuropathy.[1,2] The neurological consequences of diabetes mellitus in the central nervous system (CNS) have gained attention most recently.[3,4] Hippocampal damage causes episodic memory deficit and prevents the elaboration of spatial relational memory. Hippocampus is one of the

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central structures of the CNS where diabetic complications can alter its neuronal structure.[5,6] Depression and anxieties are about twofold higher in diabetic patients than in the general population. Increasing evidence points toward an association between diabetes mellitus and deficit in learning and memory.[5,7] Findings of the research and evidences in the literature revealed reduced neurogenesis in the dentate gyrus of diabetic hippocampus[8] and decreased dendritic arborization.[9]

Stress exposure triggered fear memory and anxiety, and decreased the hippocampal-dependent memory performance in mice.[10,11] Numerous studies revealed that the stress exposure causes alterations in synaptic function and plasticity[12] in dendritic remodeling of hippocampal neurons.[13]

Environmental enrichment (EE) exposure treatment is widely used for managing fearfulness, abnormal behaviors, and stress in laboratory animals.[14] Exposure to EE was known to enhance spatial memory abilities and synaptic strength in the hippocampus of the brain.[15] EE exposure is known to influence the CNS at the functional, anatomical, and molecular levels, both during the critical period and during adulthood.[16] It is believed that complex environmental stimuli induce structural changes in the neuronal network to enhance synaptic efficacy and improve learning and memory.[17]

METHODS

Animal groups

Male albino rats of Wistar strain, 4-5 weeks old, were housed under 12:12 h dark: light environment in institutional animal house. Animals were given water *ad libitum* and the standard pellet (Hindustan Lever, India) was used for feeding. The study was approved by the institutional animal ethical committee (IAEC/KMC/07/2007-2008). Animals were maintained in accordance with the guidelines of Government of India for use of laboratory animals. Rats in the experiment were divided into normal control (NC), vehicle control (VC), diabetes (DI), diabetes + stress (DI + S), diabetes + EE (DI + E), and diabetes + stress + EE (DI + S + E) groups (n = 8 in each group). Rats in the VC group were given vehicle solution (citric acid buffer, pH 4.5) and were continued in their home cage throughout the experimental period (Day 30).

Preparation of fresh STZ solution and creating an experimental hyperglycemic model

Streptozotocin (STZ) powder (Sigma, St. Louis, MO, USA) was dissolved in citrate buffer (pH 4.5). STZ solution was maintained in ice-cold tray to prevent decomposition by external temperature. Rats in DI, DI + S, DI + E, and DI + S + E groups were fasted overnight and made hyperglycemic with a single intraperitoneal injection of STZ solution at a dose of 40 mg/kg body weight. They were fed with 5% glucose solution to overcome drug-induced hypoglycemia.

Blood glucose levels and body weight measurement

Blood was collected from orbital plexus and tail vein, and glucose levels were measured with a glucometer (Accu-Chek Advantage Glucose Monitor) before STZ injection, 2 days after STZ injection, and on the day of sacrifice (Day 30). Rats having blood glucose levels more than 225 mg/dl (after STZ injection) were considered as diabetic and were used for the experimentation. Body weight of all the rats in different groups was verified at the beginning of experiment, 2 days after STZ injection, and before sacrifice (Day 30).

Restraint stress method

A wire mesh restrainer cage was prepared with 12 cm length, 5.5 cm height, and 5.5 cm width. It had a wooden base and a stainless steel wire mesh restrainer provided with air holes for ventilation to the rat. The restraint stress paradigm was initiated for rats in DI + S and DI + S + E groups from the 2nd day of STZ injection. Rats were made to undergo 6 h of stress period daily. After the stress session, rats were reserved in their home cages for access to food and water. After the last stress session on Day 30, the rats were sacrificed and brains were removed for fixation.

EE method

EE wood cage was made with dimensions of 50 cm length, 50 cm width, and 29 cm height. To allow the animals to explore and interact with many different objects, the cage was filled with a large variety of objects such as rotating wheels, plastic tubes, and toys of different dimensions. EE exposure was initiated for the rats in DI + E and DI + S + E groups daily for 6 h. The number and types of toys or objects were different every day to create a different environment for the animals. Three to four animals were housed in the enriched cages at one time for free movement and social interaction and were given access to food and water in the cage. EE exposure was continued till the end of the experimental period (Day 30).

Microscopic study of rat hippocampus

Tissue fixation, processing, and sectioning

Rats were sacrificed by perfusion method (by pumping 100 ml of heparinized saline and 300 ml of 4% paraformaldehyde to the left ventricle of the heart) and the brains were fixed in the paraformaldehyde solution for 48 h. Brain

tissues were processed through different grades of alcohol (50% for 24 h, 70% for 24 h, 90% for 12 h, and 100% for 12 h) and were immersed in xylene for 1-2 h. Paraffin blocks were made in an embedding bath. Using a rotary microtome (Leica RM 2245, Nussloch, Germany), coronal sections of 5 μm thickness were cut from the dorsal part of hippocampus. Twenty-five to thirty sections from each animal were mounted serially on air-dried gelatinized slides.

Cresyl violet staining procedure

Brain sections were processed in xylene, ascending grades of alcohol (absolute alcohol: 1 min, 90% alcohol: 2 min, 70% alcohol: 2 min, and 50% alcohol: 2 min), and distilled water. Sections were stained with 0.1% cresyl violet stain for 25-30 min at 60 C and were mounted with Distyrene Plasticizer Xylene

Quantification of neurons

Neuron quantification was carried out with help of a light microscope under –40 (Magnus; Olympus Pvt Ltd, New Delhi, India). Ten sections from each rat were selected for quantification. The numbers of neurons that survived with a clear and distinct nucleus in the CA1, CA3, and DH regions of the hippocampus were quantified [Figure 1]. Neurons that were darkly stained and had shrunken cell body with irregular nuclei were avoided for quantification.

Data analysis

Data were analyzed using analysis of variance (ANOVA) with Bonferroni's multiple comparison test as *post-hoc* test (GraphPad Prism 2.01 software, GraphPad Software, Inc. USA). Values are represented as mean ± standard error of mean (SEM), with a probability level of $p < 0.05$ as the criterion for statistical significance expression.

RESULTS

Blood glucose levels

Blood glucose levels were not significantly different in all the experimental groups at the commencement of the experiment (NC: 93.2 ± 4.12, VC: 91.16 ± 5.22, DI: 92.07 ± 3.10, DI + S: 90.66 ± 4.18, DI + E: 93.44 ± 2.33, and DI + S + E: 92.24 ± 3.54). Two days after the STZ injection, the blood glucose levels of rats in the diabetic groups were elevated and were significant ($p < 0.001$) in comparison to the levels of rats in the NC group (blood glucose more than 250 mg/dl; NC: 94.25 ± 4.26, VC: 92.48 ± 3.67, DI: 306.63 ± 33.16, DI + S: 267.54 ± 30.29, DI + E: 314.6 ± 27.06, and DI + S + E: 277.82 ± 33.56). The blood glucose levels were significantly increased ($p < 0.001$) in diabetic groups in comparison to the NC group by the end of the experimental period (Day 30) (NC: 92.33 ± 4.2, VC: 94 ± 5.12, DI: 455 ± 36.23, DI + S: 398.2 ± 25.22, DI + E: 472.44 ± 37.8, and DI + S + E: 555.42 ± 45.32).

Body weight

The body weights of rats in all the groups did not differ significantly in the beginning of the experiment (NC: 92.14 ± 3.11, VC: 93.6 ± 4.02, DI: 91.82 ± 4.4, DI + S: 92.16 ± 3.5, DI + E: 93.35 ± 3.72, and DI + S + E: 94.55 ± 4.15). A decrease in body weight was observed in the DI, DI + S, DI + E, and DI + S + E groups on the 2nd day of the experiment (after STZ injection) compared to rats in the NC group, but was not significant (NC: 108.22 ± 2.5, VC: 105.7 ± 2.66, DI: 98.5 ± 3.25, DI + S: 103.4 ± 3.55, DI + E: 102 ± 2.2, and DI + S + E: 99.22 ± 3.16). A highly significant ($p < 0.001$) decrease in body weight was observed in DI, DI + S, DI + E, and DI + S + E groups compared to NC group rats before sacrifice (Day 30) (NC: 218.5 ± 8.5, VC: 212.3 ± 6.13, DI: 87 ± 4.11, DI + S: 73 ± 5.62, DI + E: 88.2 ± 8.3, and DI + S + E: 71.59 ± 6.63).

Effect of EE on diabetic rats and on the combined action of diabetes and stress on rat hippocampus [Figures 2-6]

Surviving neurons in CA1 of the hippocampus

DI (34.06 ± 3.2) and DI + S (14.03 ± 3.12) groups showed a significant decrease ($p < 0.001$) in the number of surviving neurons compared to NC group (53.64 ± 2.96), and a significant increase ($p < 0.001$) in the number of surviving neurons was observed in DI + E (42.3 ± 3.66) and DI + S + E (29.69 ± 4.47) groups compared to DI and DI + S groups, respectively [Figures 2 and 5].

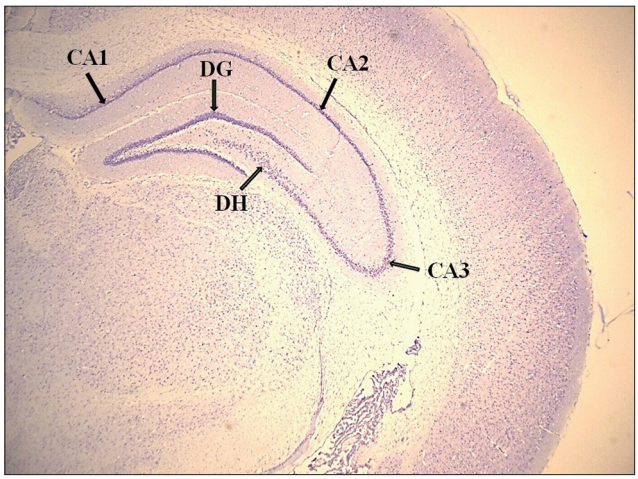

Figure 1: Photomicrograph showing the subdivisions of rat hippocampus (×4, cresyl violet stain) (CA1, cornu ammonis 1; CA2, cornu ammonis 2; CA3, cornu ammonis 3; DH, dentate hilus; DG, dentate gyrus).

Surviving neurons in CA3 of the hippocampus

A significant decrease ($p < 0.001$) in the number of surviving neurons in DI rats (36.1 ± 3.62) was observed compared to NC group (62.1 ± 3.34). Rats in the DI + S group (20.27 ± 4.09) also showed a significant decrease ($p < 0.001$) in the number of surviving neurons compared to NC group rats. Rats in DI + E group (46.73 ± 4.74) showed a significant ($p < 0.001$) increase in the number of surviving neurons compared to the rats in the DI group. DI + S + E (36.73 ± 3.89) group rats showed a significant ($p < 0.001$) increase in the number of surviving neurons when compared to the DI + S group rats [Figures 3 and 6].

Surviving neurons in DH of the hippocampus

Compared to NC rats (21.11 ± 1.03), DI (9.83 ± 2.02) and DI + S (6.4 ± 1.21) rats showed a significant decrease ($p < 0.001$) in the number of surviving neurons. DI + E rats (17.03 ± 2.19) showed a significant ($p < 0.001$) increase in the number of neurons compared to the DI rats. DI + S + E (12.23 ± 2.36) rats also showed a significant ($p < 0.001$) increase in the number of neurons when compared to the DI + S group rats [Figures 4 and 7].

DISCUSSION

Uncontrolled hyperglycemia in diabetes can lead to altered neurochemical profiles, cerebral blood flow with structural abnormalities, which may involve direct neuronal damage in the brain.[18] Within the CNS, the hippocampus is considered as a special target for alterations associated with diabetes.[19] The process of nerve injury in STZ-induced diabetes mellitus is very similar to the nerve injury in human diabetic neuropathy.[20] Diabetes results in increased formation of free radicals by cellular glucose autoxidation with decreased antioxidant potential. In animals and *in vitro* models of diabetes, hyperglycemia, along with the other complications of diabetes causes damage by activating the key pathways of glucose metabolism such as advanced glycation end product formation, increased hexosamine pathway, and superoxide overproduction.[2,21,22] Free radical production results in neuronal injury by initiating apoptosis. In addition to the oxidative stress, impaired insulin signaling pathway and reduced level of brain-derived neurotrophic factors may also be involved in the development of neuropathy.[23,24]

Restrain stress exposure decreases food intake and body weight gain in rats.[25,26] Excessive cortisol produced under stress causes damage to arteries in the brain, with subsequent increased risk of stroke and damage to the brain regions by initiating apoptosis.[27,28] In the aftermath of stress[29] and recurrent depression,[30] the hippocampus undergoes atrophy. Previous studies had shown decreased cell proliferation in the hippocampus in post-stress period.[31]

According to Reagan *et al*. (1999), hippocampus of hyperglycemic rat is extremely susceptible to additional stressful events, which in turn can lead to severe hippocampal damage.[3] Accordingly, the present study was designed to assess

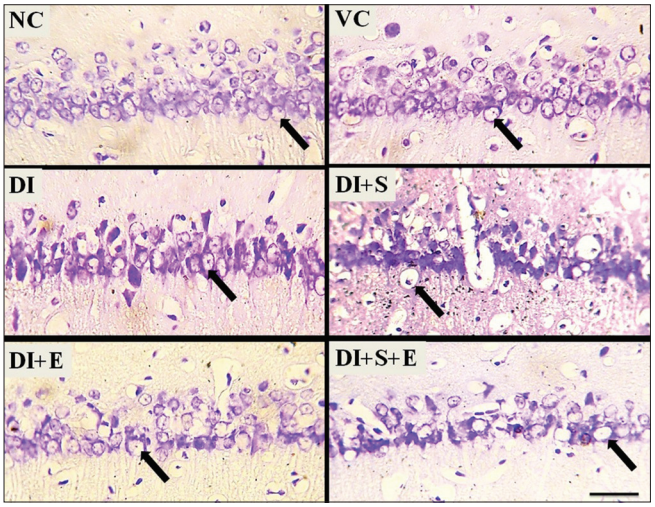

Figure 2: Photomicrograph of CA1 region of hippocampus (×40, cresyl violet stain) showing the effect of environmental enrichment on diabetes and combined actions of diabetes and stress on the pyramidal neurons. Decreased number of surviving neurons (marked with arrow, clear rounded cells having distinct nucleus) and cell shrinkage were observed in DI and DI + S groups compared to NC group. A significantly increased ($p < 0.001$) number of surviving neurons was observed in DI + E and DI + S + E groups compared to DI and DI + S group rats, respectively. Bar 20 µm.

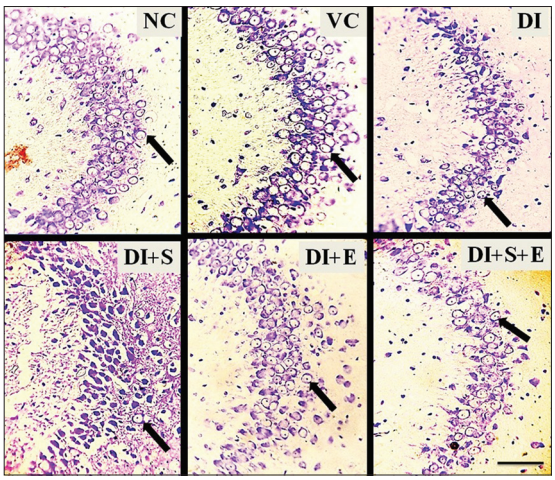

Figure 3: Photomicrograph of CA3 region of hippocampus (×40, cresyl violet stain) showing the effect of environmental enrichment on diabetes and combined actions of diabetes and stress on the pyramidal neurons. Decreased number of surviving neurons (marked with arrow, clear rounded cells having distinct nucleus) and cell shrinkage were observed in DI and DI + S groups compared to NC group. A significantly increased ($p < 0.001$) number of surviving neurons was observed in DI + E and DI + S + E groups compared to DI and DI + S group rats, respectively. Bar 20 µm.

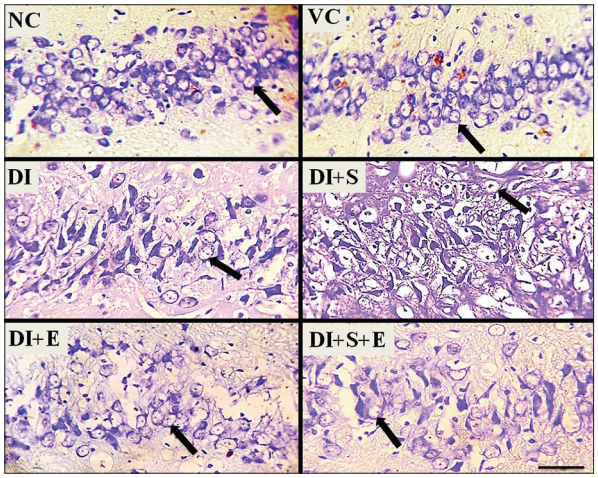

Figure 4: Photomicrograph of dentate hilus of hippocampus (×40, cresyl violet stain) showing the effect of environmental enrichment on diabetes and combined actions of diabetes and stress on the pyramidal neurons. Decreased number of surviving neurons (marked with arrow, clear rounded cells having distinct nucleus) and cell shrinkage were observed in DI and DI + S groups compared to NC group. A significantly increased ($p < 0.001$) number of surviving neurons was observed in DI + E and DI + S + E groups compared to DI and DI + S group rats, respectively. Bar 20 µm.

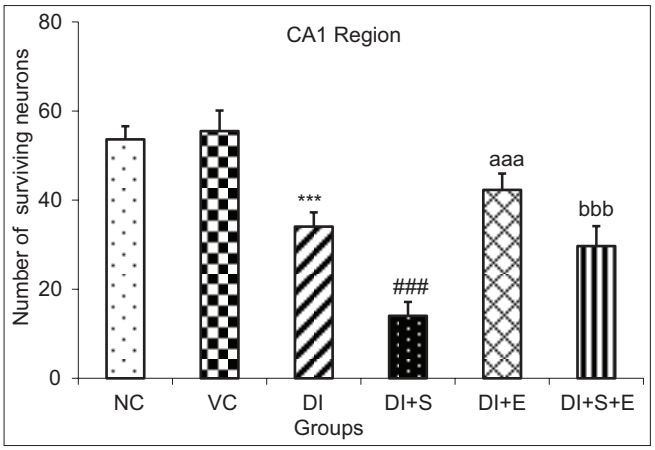

Figure 5: Bar graph showing the number of survived neurons in the CA1 region of hippocampus of rat brain. Bars represent mean ± SEM (NC vs. DI, $^{***}p < 0.001$; NC vs. DI + S, $^{###}p < 0.001$; DI vs. DI + E, $^{aaa}p < 0.001$; DI + S vs. DI + S + E, $^{bbb}p < 0.001$).

the damage inflicted to hippocampal neurons of experimental diabetes with restraint stress. Highly significant Nissl body damage was seen in the subregions of the hippocampus (CA1, CA3, and DH) in DI + S rats compared to NC group rats. Significant neuronal shrinkage and degenerated nuclei in the DI + S rats might be due to alterations in the volume of the hippocampus caused by discharge of serotonin and adrenal steroid to increase the effectiveness of stressful events.[32-34]

Colorful objects of different shapes, sizes, textures, and bold shapes are known to stimulate brains to the greatest

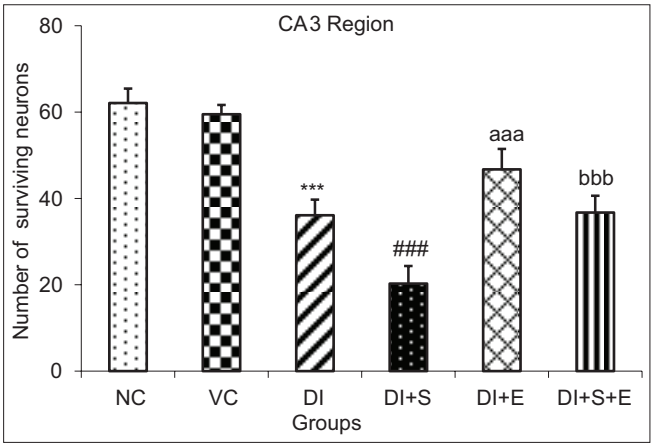

Figure 6: Bar graph showing the number of survived neurons in the CA3 region of hippocampus of rat brain. Bars represent mean ± SEM (NC vs. DI, $^{***}p < 0.001$; NC vs. DI + S, $^{###}p < 0.001$; DI vs. DI + E, $^{aaa}p < 0.001$; DI + S vs. DI + S + E, $^{bbb}p < 0.001$).

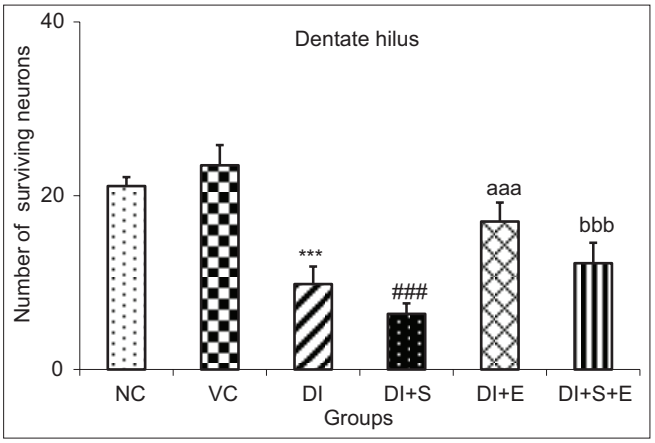

Figure 7: Bar graph showing the number of survived neurons in the dentate hilus region of hippocampus of rat brain. Bars represent mean ± SEM (NC vs. DI, $^{***}p < 0.001$; NC vs. DI + S, $^{###}p < 0.001$; DI vs. DI + E, $^{aaa}p < 0.001$; DI + S vs. DI + S + E, $^{bbb}p < 0.001$).

extent possible. They provide entertainment and enhance cognitive behavior and stimulate creativity.[35] EE exposure brings about changes from the molecular to structural level for improving the functions of intact and lesioned brain.[36,37] EE exposure assists in the survival of brain networks by accelerating synaptogenesis, axon sprouting,[38-40] and helps in the survival and differentiation of newborn cells of stressed rat hippocampus.[41] In a study by Beauquis *et al*. (2010), 10 days of EE exposure to diabetic mice resulted in increased neurogenesis, vascular network, and dendritic complexity in the hippocampus.[42]

Relief from uncontrolled diabetes and stressors is thought to be absolutely necessary in order to improve the overall condition of hippocampal neurons. Considering this fact, we exposed the rats in DI and DI + S groups to EE. Upon EE exposure, degeneration of neurons was prevented

in DI + E and DI + S rats. The observed changes in the number of surviving neurons of DI and DI + S hippocampus after EE exposure might be due to the increased neural trophic factors such as brain-derived neurotrophic factor, nerve growth factor, and vascular endothelial growth factor,[43-45] which are involved in structural reorganization of brain after an injury caused by the uncontrolled hyperglycemic and stress conditions.

Conclusions

EE exposure significantly reduced the quantity of neuronal damage caused by hyperglycemic effects of diabetes as well as by the combined actions of diabetes and stress to the neurons of hippocampus. The results of the study may be helpful in correlating and understanding the complications of diabetes and stress on the hippocampal neurons of diabetic patients and in the prevention of the neuronal damage with the help of EE exposure.

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REFERENCES

1. Brown MJ, Asbury AK. Diabetic neuropathy. Ann Neurol 1984;15:2-12.
2. Tripathi BK, Srivastava AK. Diabetes Mellitus: Complications and Therapeutics. Med Sci Monit 2006;12:130-47.
3. Reagan LP, Magarinos AM, McEwen BS. Neurological changes induced by stress in streptozotocin diabetic rats. Ann N Y Acad Sci 1999;893:126-37.
4. Revsin Y, Saravia F, Roig P, Lima A, de Kloet ER, Homo-Delarche F, *et al*. Neuronal and astroglial alterations in the hippocampus of a mouse model for type 1 diabetes. Brain Res 2005;1038:22-31.
5. Jackson-Guilford J, Leander JD, Nisenbaum LK. The effect of streptozotocin induced diabetes on cell proliferation in the rat dentate gyrus. Neurosci Lett 2000;293:91-4.
6. Pamidi N, Satheesha Nayak B. Effect of Streptozotocin induced diabetes on rat hippocampus. Bratisl Lek Listy 2012;113:583-8.
7. Biessels GJ, Kappelle AC, Bravenboer B, Erkelens DW, Gispen WH. Cerebral function in diabetes mellitus. Diabetologia 1994;37:643-50.
8. Zhang WJ, Tan YF, Yue JT, Vranic M, Wojtowicz JM. Impairment of hippocampal neurogenesis in streptozotocin-treated diabetic rats. Act Neurol Scand 2008;117:205-10.
9. Martinez-Tellez R, Gomez-Villalobos Mde J, Flores G. Alteration in dendritic morphology of cortical neurons in rats with diabetes mellitus induced by streptozotocin. Brain Res 2005;1048:108-15.
10. Chen Q, Tang M, Mamiya T, Im HI, Xiong X, Joseph A, *et al*. Bi-directional effect of cholecystokinin receptor-2 overexpression on stress-triggered fear memory and anxiety in the mouse. PLoS One 2010;5:e15999.
11. Beracochea D, Tronche C, Coutan M, Dorey R, Chauveau F, Pierard C. Interaction between diazepam and hippocampal corticosterone after acute stress: Impact on memory in middle-aged mice. Front Behav Neurosci 2011;5:14.
12. Kim JJ, Diamond DM. The stressed hippocampus, synaptic plasticity and lost memories. Nat Rev Neurosci 2002;3:453-62.
13. Magarinos AM, McEwen BS. Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: Involvement of glucocorticoid secretion and excitatory amino acid receptors. Neuroscience 1995;69:89-98.
14. Fairhurst GD, Frey MD, Reichert JF, Szelest I, Kelly DM, Bortolotti GR. Does environmental enrichment reduce stress? An integrated measure of corticosterone from feathers provides a novel perspective. PLoS One 2011;6:e17663.
15. Kempermann G, Kuhn HG, Gage FH. More hippocampal neurons in adult mice living in an enriched environment. Nature 1997;386:493-5.
16. Baroncelli L, Braschi C, Spolidoro M, Begenisic T, Sale A, Maffei L. Nurturing brain plasticity: Impact of environmental enrichment. Cell Death Differ 2010;17:1092-103.
17. Huang FL, Huang KP, Wu J, Boucheron C. Environmental enrichment enhances neurogranin expression and hippocampal learning and memory but fails to rescue the impairments of neurogranin null mutant mice. J Neurosci 2006;26:6230-7.
18. Hernandez-Fonseca JP, Rincon J, Pedreanez A, Viera N, Arcaya JL, Carrizo E, *et al*. Structural and ultrastructural analysis of cerebral cortex, cerebellum, and hypothalamus from diabetic rats. Exp Diabetes Res 2009;2009:329632.
19. Saravia F, Revsin Y, Lux-Lantos V, Beauquis J, Homo-Delarche F, De Nicola AF. Oestradiol restores cell proliferation in dentate gyrus and subventricular zone of streptozotocin-diabetic mice. J Neuroendocrinol 2004;16:704-10.
20. Reagan LP, McEwen BS. Diabetes, but not stress, reduces neuronal nitric oxide synthase expression in rat hippocampus: Implications for hippocampal synaptic plasticity. Neuroreport 2002;13:1801-4.
21. Zaltzberg H, Kanter Y, Aviram M, Levy Y. Increased plasma oxidizability and decreased erythrocyte and plasma antioxidative capacity in patients with NIDDM. Isr Med Assoc J 1999;1:228-31.
22. Rahimi R, Nikfar S, Larijani B, Abdollahi M. A review on the role of antioxidants in the management of diabetes and its complications. Biomed Pharmacother 2005;59:365-73.
23. Wisniewski K, Fedosiewicz-Wasiluk M, Holy ZZ, Car H, Grzeda E. Influence of NMDA, a potent agonist of glutamate receptors, on behavioral activity in 4-week streptozotocin-induced diabetic rats. Pol J Pharmacol 2003;55:345-51.
24. Dagon Y, Avraham Y, Link G, Zolotarev O, Mechoulam R, Berry EM. The synthetic cannabinoid HU-210 attenuates neural damage in diabetic mice and hyperglycemic pheochromocytoma PC12 cells. Neurobiol Dis 2007;27:174-81.
25. Krahn DD, Gosnell BA, Majchrzak MJ. The anorectic effects of CRH and restraint stress decrease with repeated exposures. Biol Psychiatry 1990;27:1094-102.
26. Dhungel S, Bhattacharya S, Shrestha RN. Effect of restraint stress on the growth rates of young wistar rats. J Nepal Health Res Counc 2006;4:10-6.
27. Yusim A, Ajilore O, Bliss T, Sapolsky R. Glucocorticoids exacerbate

insult-induced declines in metabolism in selectively vulnerable hippocampal cell fields. Brain Res 2000;870:109-17.

28. Crochemore C, Lu J, Wu Y, Liposits Z, Sousa N, Holsboer F, *et al*. Direct targeting of hippocampal neurons for apoptosis by glucocorticoids is reversible by mineralocorticoid receptor activation. Mol Psychiatry 2005;10:790-8.
29. Bremner JD, Randall P, Scott TM, Bronen RA, Seibyl JP, Southwick SM, *et al*. MRI-based measurement of hippocampal volume in patients with combat-related posttraumatic stress disorder. Am J Psychiatry 1995;152:973-81.
30. Sheline YI, Wang PW, Gado MH, Csernansky JG, Vannier MW. Hippocampal atrophy in recurrent major depression. Proc Natl Aca dSci USA 1996;93:3908-13.
31. Mineur YS, Belzung C, Crusio WE. Functional implications of decreases in neurogenesis following chronic mild stress in mice. Neuroscience 2007;150:251-9.
32. McEwen BS. Mood disorders and allostatic load. Biol Psychiatry 2003;54:200-7.
33. McEwen BS. Physiology and neurobiology of stress and adaptation: Central role of the brain. Physiol Rev 2007;87:873-904.
34. McEwen BS, Milner TA. Hippocampal formation: Shedding light on the influence of sex and stress on the brain. Brain Res Rev 2007;55:343-55.
35. Van Praag H, Kempermann G, Gage FH. Neural consequences of environmental enrichment. Nat Rev Neurosci 2000;1:191-8.
36. Nilsson M, Perfilieva E, Johansson U, Orwar O, Eriksson PS. Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory. J Neurobiol 1999;39:569-78.
37. Keyvani K, Sachser N, Witte OW, Paulus W. Gene expression profiling in the intact and injured brain following environmental enrichment. J Neuropathol Exp Neurol 2004;63:598-609.
38. Dobkin BH. Functional rewiring of brain and spinal cord after injury: The three Rs of neural repair and neurological rehabilitation. Curr Opin Neurol 2000;13:655-9.
39. Nudo RJ, Plautz EJ, Frost SB. Role of adaptive plasticity in recovery of function after damage to motor cortex. Muscle Nerve 2001;24:1000-19.
40. Ip EY, Giza CC, Griesbach GS, Hovda DA. Effects of enriched environment and fluid percussion injury on dendritic arborization within the cerebral cortex of the developing rat. J Neurotrauma 2002;19:573-85.
41. Veena J, Srikumar BN, Mahati K, Bhagya V, Raju TR, Shankaranarayan Rao BS. Enriched environment restores hippocampal cell proliferation and ameliorates cognitive deficits in chronically stressed rats. J Neurosci Res 2009;87:831-43.
42. 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 2010;5:e13993.
43. Pham TM, Ickes B, Albeck D, Soderstrom S, Granholm AC, Mohammed AH. Changes in brain nerve growth factor levels and nerve growth factor receptors in rats exposed to environmental enrichment for one year. Neuroscience 1999;94:279-86.
44. Wang Y, Jin K, Mao XO Xie L, Banwait S, Marti HH, Greenberg DA. VEGF overexpressing transgenic mice show enhanced post-ischemic neurogenesis and neuromigration. J Neurosci Res 2007;85:740-7.
45. Sun H, Zhang J, Zhang L, Liu H, Zhu H, Yang Y. Environmental enrichment influences BDNF and NR1 levels in the hippocampus and restores cognitive impairment in chronic cerebral hypoperfused rats. Curr Neurovasc Res 2010;7:268-80.