Original Article

Alpha7 Nicotinic Acetylcholine Receptor Down Regulation Impairs Mitochondrial Function in Streptozotocin-induced Sporadic Alzheimer's Disease Model in Rats

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ABSTRACT

Background/Aim: Alzheimer's disease (AD), a type of neurodegenerative disorder, possesses significant memory loss as one of the cardinal manifestations. The pathophysiology of AD includes increased accumulation of Aβ, degeneration of cholinergic activity and mitochondrial dysfunction. Nicotinic acetylcholine receptors (nAChRs) especially alpha7 nicotinic acetylcholine receptors (α7 nAChRs) are widely distributed in brain and associated with memory function. Further, we explored the correlation between the mitochondrial dysfunction and the expression level of α7 nAChRs in STZ challenged brain regions of rats. **Materials and Methods:** The STZ group rats received intracerebroventricular infusion of STZ (3 mg/kg) on D-1 and D-3 of experimental design of 18 days. Behavioral parameters ware investigated using MWM and Y-maze test paradigm. Further, biochemical analysis was assessed in all three regions of rats. **Results:** STZ administration caused significant impairment in memory and learning of rats MWM and Y-maze test paradigm. There was significant decrease in level expression of α7 nAChRs and cholinergic functions in terms of elevated AChE activity and decreased ACh level and ChAT activity in rat hippocampus, pre-frontal cortex and amygdala. Further, STZ administration significantly attenuated the mitochondrial function, integrity and bioenergetics in all the selected brain regions. Interestingly, the intracerebroventricular infusion of STZ increased Aβ level in all the rat brain regions. **Conclusion:** The α7 nAChR down regulation may form a basis to cognitive deficits along with cholinergic dysfunction, Aβ accumulation and mitochondrial dysfunction in memory sensitive rat brain regions. Thus, α7 nAChR could be an alternative and potential target in the management of AD.

Key words: Alzheimer's disease, Streptozotocin, α7 nAChR, Mitochondria, Acetylcholine, Hippocampus.

INTRODUCTION

Alzheimer's disease (AD), a type of neurodegenerative disorder, possesses significant memory loss as one of the cardinal manifestations. The pathophysiology of AD includes increased accumulation of amyloid beta (Aβ), degeneration of cholinergic activity, dysregulation in the calcium homeostasis and mitochondrial dysfunction.[1,2] It is expected that approximately 115 million people will be affected with AD throughout the globe by 2050.[3,4] Several drugs have been considered in the management of AD however, their use is restricted due to evidence of severe adverse effects.[5] Hence, there is a need to develop novel therapeutic target to manage the progress of AD.

The nicotinic acetylcholine receptors (nAChRs) especially alpha7 (α7) are expressed throughout the brain and they are also associated in the process of memory formations. Utsugisawa *et al.* reported down regulation of the α7 nAChR in the brain of the subjects in postmortem study.[6] It has

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been well reported that progressive loss of cognitive ability is associated with early deficits in the α7 nAChRs.[7] Literature review suggests that the α7 nAChRs plays a critical role in the physiology of Aβ accumulation and also contribute in the pathophysiology of AD.[8,9] Aβ modulates postsynaptic nAChRs function especially α7 nAChR.[10] It has been reported that neuronal inflammation may contribute to the reduction in α7 nAChR density in rodent brains and is associated with Aβ deposition and memory impairments.[11,12] On contrary, the activation of α7 nAChR attenuates Aβ toxicity and exerts neuroprotection in the experimental studies.[13,14] The activation of α7 nAChR also facilitates the activity of acetylcholine (ACh) in brain.[15] Further, selective α7 nAChR agonist improves memory formation/function in the rodents.[16,17] It has been reported that the α7 nAChRs in the plasma membrane influence Ca^{2+} accumulation, voltage-dependent anion channel (VDAC)-mediated Ca^{2+} transport and mitochondrial permeability and regulate the mitochondrial-dependent cell apoptosis.[18] It is also suggested that the α7 nAChR could possess a major clinical and pharmacological implications in the management of AD.[19]

It has been observed that the intracerebroventricular (ICV) injection of streptozotocin (STZ) alters behavioral functions including memory impairements[20] and neurochemical functions[21,22] similar to AD condition. It is demonstrated that ICV-STZ leads to mitochondrial dysfunction[23] with deficiency in cholinergic activity[24] in AD-like rodents. Tota *et al.* reports significant reduction in the expression level of α7 nAChRs with concomitant dysfunction in mitochondria and cholinergic activity in the STZ-challenged rodent brains.[25] However, there is no report on the brain α7 nAChRs level in STZ-challenged animals.

Therefore, present study was undertaken to explore the level of expression of α7 nAChRs and, function, integrity and bioenergetics of mitochondria in the memory-sensitive brain areas of STZ-challenged rats.

MATERIALS AND METHODS

Animals

Swiss albino wistar male rats (200 ± 20 g) were collected from the animal house of GLA University, Mathura and Uttar Pradesh. The rats were divided into three equal-sized groups (n=6) and housed at ambient condition (temperature 25±1°C, relative humidity 50±5% and light/dark cycle 12:12 h) in poly-acrylic cages. Animals received their standard pellet feed (Lipton India, Ltd., Mumbai) and water *ad libitum* in the experimental study. All the animals were fasted for 14 ± 1 h before experimentation, but water was supplied in sufficient quantity. The study deign was approved from the institute for experiment on animals (GLAIPR/CPCSEA/IAEC/P'Col/2016/01).

Reagents and Chemicals

All the used chemicals in the experimentation were of analytical grade and available commercially. STZ, TMRM and Amplex red assay kit for acetylcholinesterase (AChE) and ACh were procured from Sigma (St. Louis, MO, USA) while beta-actin was purchased from Abcam Plc., Cambridge, USA.

Experimental Design

The experimental design consisted of eighteen days study (Figure 1). Rats were randomly allocated into 3 groups (Control, Sham and STZ) having 6 animals in each group. ICV-STZ was injected on Day (D)-1 and D-3 of experimental protocol only to STZ group rats. Sham group animals received artificial cerebrospinal fluid (aCSF). The Morris Water Maze (MWM) test paradigm was carried out for 5 successive days (from D-14 to 18) of the experimental design. Further, Y-maze test paradigm was carried out on D-18 with a one hour of lag between two test paradigms. All the behavior parameters were record and computed with ANY-Maze™ video-tacking software (Version-4.69, USA). After that decapitation method was used to sacrifice all the rats. The brain were removed and micro-dissected into hippocampus (HIP), prefrontal cortex (PFC) and amygdale (AMY) and used for further biochemical estimation.[26,27]

ICV Injection of STZ

Overnight fasted rat was anesthetized with intraperitoneal injection of sodium pentobarbital (40 mg/kg). After that an incision was made on the scalp and the head was positioned in a stereotaxic apparatus (Stoelting, USA). On each sides of lateral ventricle, two holes (1.5 mm depth) were drilled (0.8 mm posterior, ±1.5 mm medio-lateral relative to bregma and 3.8 mm drso-ventral below to dura).[26] Freshly prepared STZ solution in aCSF (47 mM NaCl; 2.9 mM KCl; 1.6 mM

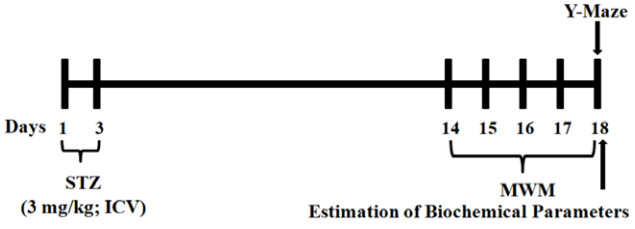

Figure 1: Diagrammatic representation of detailed experimental schedule.

$MgCl_2$; 1.7 mM $CaCl_2$ and 2.2 mM dextrose) was injected through Hamilton syringe using polyethylene tube into both side of lateral ventricle over a period of 5 min on day first followed by day third (3 mg/kg; 5 µL each site at time lag of 5 min).[28,29] aCSF (10 µl) was injected at a volume of 5 µl into each site of the lateral ventricle of Sham animals. Quintessential Stereotaxic Injector (Stoelting, USA) was used for all microinjections.

Assessment of STZ-induced Memory Impairment in Different Behavior Tests

Learning and Memory in Morris Water Maze Test Model

Learning and memory of all the animals were quantified according to the MWM test procedure because it is one of the most widely used animal model to access memory and learning.[30] Briefly, a circular black large pool of water tank (diameter 150 cm; height 60 cm) filled with water up to 30 CM was included in the task to access learning and memory of animals. The water pool temperature was maintained at 26±1°C. A water tank was partitioned into 4 equal quadrants with equal arc. A hidden platform (diameter 4.5 cm) was placed below 1.5 cm of water surface in the center of target quadrant. Video tracking system was used to record the behavior of each animal. Randomly, each animal was placed in water tank for four consecutive trails (with the gap interval of 5 min between each trail) for 120 s in every day for four days from D-14 to D-17 of experimental protocol to search hidden platform and escape latency was recorded. On D-18, the hidden platform was taken out from the water pool during experimental procedure and tine spent in target quadrant and in each quadrant to search out platform was recorded. Swimming speed and percentage of total distance travelled in target quadrant were also recoded. Time spent in target quadrant was considered as marker of memory retrieval and memory building.

Spontaneous Alteration Behavior in Y-Maze Test Paradigm

The Y-maze test paradigm is considered to assess working memory of rodents. The spontaneous alteration behavior (SAB) was considered as index of working memory. Standard protocol was followed to observe SAB.[31] Y-Maze (apparatus consist of three wooden arms labeled as A, B and C, 3 cm wide and 40 cm long with 12 cm of wall height) test paradigm was performed on lost day (D-18) of experimental protocol. Randomly each animal was placed at midpoint of T-maze apparatus for 8 min. During this time period rat was made free to move and successive entry sequence of ABC, BCA or CAB (but not ABA) and total number of arm entries were noted down. After each experiment the arms of Y-maze were wiped to remove resting odor.

Evaluation of Effect of STZ on Cholinergic System

Sample Preparation

HIP, PFC and AMY regions of brain were identified and homogenized in 01 mL of perchloric acid (0.1 M) separately by a homogenizer. The homogenates were transferred into polypropylene tubes followed by addition of potassium acetate (4 M; 50µL) to maintain the pH to 4.0. Then resultant preparations were centrifuged at 4000Xg for 15 min.[32]

Assay of Choline Acetyltransferase (ChAT) Activity

ChAT activity was analyzed spectrophotometrically (λ_{max} = 450 nm) with an enzyme-linked immunosorbent assay (ELISA) kit (SEB929Mu; Hubei, China) as directed by manufacturer and results were expressed as n mol/hr/mg protein.

Quantification of ACh

The level of ACh in discrete rat brain regions was quantified using Amplex red assay kit (Molecular Probes, inc., USA) as per standard protocol illustrated by Zoukhri and Kublin.[33] In brief, homogenized tissue and 0.1 mL of 10 µM H_2O_2 (as control) were collected in three polypropylene tubes separately followed by addition of 0.1 mL of buffer (50 mM Tris-hydrochloric acid, pH 7.5) previously containing choline oxidase (0.2 U/mL), AChE (10 U/mL), Amplex red reagent (0.2 M) and horseradish peroxidase (2 U/mL) into each tubes. The fluorescence of each preparation was noted using spectrophotometer (λ_{max} = 530 nm) at excitation (λ_{max} = 530 nm) and emission wavelength (λ_{max} = 590 nm). The protein content for each brain regions was estimated as per set protocol.[34]

Estimation of AChE Activity in Discrete Brain Region of Rats

To determine the AChE activity a standard protocol was followed as described in Amplex red assay kit (Molecular Probes, Inc., USA). Homogenized tissue was transferred in three separate tubes and 0.1 mL of buffer (50 mM Tris-hydrochloric acid, pH 7.5) containing Amplex red reagents (400 µM), horseradish peroxidase (2 U/mL), choline oxidase (0.2 U/mL) and ACh (100 µM) was added into each tube. After 30 min of incubation period, the fluorescence of each preparation was recorded using spectrophotometer at excitation (λ_{max} = 530 nm) and emission wavelength (λ_{max} = 590 nm). The amount of protein for each brain regions was estimated.[34]

Quantification of Cytosolic Aβ Level

The Aβ level was estimated with ELISA kit (Arigo Biolaboratories, Taiwan, ARG80939) using spectrophotometer (λ_{max} = 450 nm) as per instructions of manufacturer.

Evaluation of Effect of STZ on Mitochondrial Parameters in Discrete Brain Regions

Mitochondrial Isolation from Discrete Rat Brain Tissues

Mitochondrial isolation from HIP, PFC and AMY was performed according to standard procedure illustrated by Pedersen.[35] Thereafter, each tissue was implied for estimation of mitochondrial protein content using standard protocol of Lowry.[34]

Assessment of Mitochondrial Function in Discrete Rat Brain Region

Each rat brain tissue fraction was implied for evaluation of mitochondrial function by the method of reduction of 3-(4, 5-dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide (MTT) using spectrophotometer (λ_{max} = 595 nm).[36] The level of Formosan formed was represented as mg formazan formed/min/mg protein.

Estimation of Mitochondrial Membrane Potential (MMP) in Discrete Rat Brain Region

MMP was evaluated using flurorescent cationic dye (tetramethyl rhodamine methylester; TMRM) to estimate the integrity of mitochondria. MMP estimation was performed using spectrophotometer at an excitation (λ_{max} = 535±10 nm) and emission (λ_{max} = 580±10 nm) as illustrated by Huang.[37] The results were expressed as fluorescence intensity value/mg protein.

Evaluation of Mitochondrial Bioenergetics in Discrete Rat Brain Region

Polarography principle and Clark oxygen electrode (Hansatch Instruments Pvt. Ltd. USA) were used to evaluate extent of mitochondrial respiration. Respiratory control ratios (RCR) and ADP/O were estimated as per the standard protocol.[38]

Method of Protein Quantification

A standard protocol was used to estimate protein concentration as explained by Bradford.[39] To perform immunoblotting, discrete rat brain regions (HIP, PFC and AMY) were lysed separately in mixture of buffer and complete protease inhibitor cocktail. A standard curve of bovine serum albumin was plotted. For α7 nAChR protein, each aliquots of each sample were electrophoreses into 10% SDS-PAGE gels and transferred to polyvinyllidene fluoride membranes and probed with specific antibody. Thereafter, membrane was incubated in phosphate buffered saline (PBS) buffer having milk (5%) at room temperature for one h. After that membrane were probed with specific primary antibody (diluted with PBS, 2.5% milk and 0.1% Twen 20) for overnight at 4°C. Further membrane was incubated with rabbit anti-α7 nAChR (Abcam Plc., Cambridge, USA) primary polyclonal antibody (1:1000) for overnight. Thereafter, desired antibody was detected against α7 nAChR protein, then membrane was stripped with buffer (25 mM Glycine pH 2.0; 2% SDS) at room temperature for half an hours. Further, membrane was reprobed with anti-β-actin primary polyclonal antibody (1:500) to validate equal loading of protein for overnight. Thereafter, membrane was probed with analogous secondary antibody of β-actin. Immunoreactive band of protein was estimated with enhanced chemiluminescence (ECL) reagents by chemiluminescence. Thereafter, specific immunoreactive area was estimated by densitometric analysis with the help of Biovis gel documentation software.

Evaluation of Oxidative Stress in Discrete Rat Brain Region

Assessment of Lipid Peroxidase Activity

The lipid peroxidation (LPO) activity was evaluated calorimetrically (λ_{max} = 535 nm) using standard protocol illustrated by Ohkawa.[40] The LPO activity was measured in terms of malonaldialdehyde (MDA) concentration and represented in μmoles of MDA/mg protein.

Estimation of Level of Nitric Oxide (NO)

The NO level was evaluated using standard method.[41] The estimated result was represented in n moles of NO/mg protein.

Quantification of Catalase (CAT) Activity

In the brain, CAT is responsible for breakdown of H_2O_2 which was estimated by spectrophotometry (λ_{max} = 240 nm) using standard protocol.[42] The resulted was represented as units (U) of CAT activity/min/ mg protein.

Evaluation of Activity of Super Oxide Dismutase (SOD)

The SOD activity was estimated with the help of spectrophotometer (λ_{max} = 560 nm) using standard protocol.[43] The assay is based on production of NADH-phenazine methosulfate-nitro blue tetrazolium (NBT) formazan in discrete brain regions. The one unit (U) of enzyme was represented as 50% inhibition of NBT reduction/min/mg protein.

Data Analysis

Values were presented as mean ± standard error of mean (SEM). Repeated measures of two-way analysis of variance (ANOVA) followed by Bonferroni-*post hoc* test was performed for analysis of escape latency of rats on D-14 to D-17 in MWM test protocol. All other statistical analysis of obtained data was performed using one-way ANOVA followed by Student-Newman-Keuls *post-hoc* test to screen the significance between the groups. Value of $P < 0.05$ was taken into consideration as significance.

RESULTS

STZ Impairs Spatial Memory and Learning Ability of Rats in MWM Test Paradigm

Figure 2 (A) illustrates the representative track plot of a rat of each group during MWM test. The effect of STZ on spatial and learning ability in terms of escape latency on D-14 to D-17 (B), time spent (C) and percentage of total distance travelled (D) in the target quadrant and swimming speed (E) on D-18 are depicted in Figure 2. Two-way ANOVA showed significant differences among group ($F_{2,60}$ = 26.16) and time ($F_{3,60}$ = 63.59) in the escape latency. Further, a significant interaction was observed in between group and time in the escape latency of the animals ($F_{6,60}$ = 2.32). *post-hoc* study revealed that there were no significant differences among groups in escape latency on D-14. STZ injection increased the escape latency of the animals on D-15 compared to other group rodents. These observations were similar on D-16 and D-17.

STZ administration significantly reduced the time spent ($F_{2,15}$ = 83.84) and percentage of distance travelled in target quadrant ($F_{2,15}$ = 38.63) on D-18 of MWM test than other group rats. Moreover, there were no significant differences in swimming speed of rats ($F_{2,15}$ = 0.3.61) among the groups.

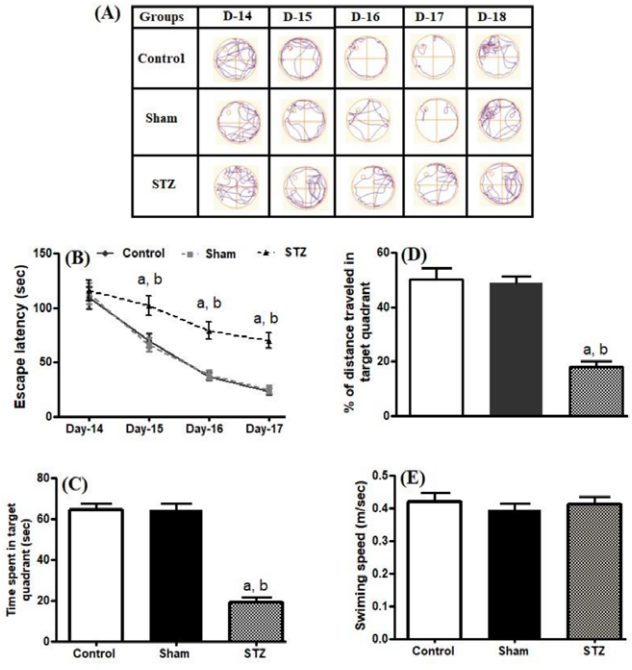

Figure 2: Representative tracking plot of an animal from all groups on D-14 to D-18 in Morris water maze (MWM) test paradigm (A). Effect of STZ on escape latency from D-14 to D-17 (B), time spent (C) and percentage of distance travelled (D) in the target quadrant and swimming speed of the animals (E) on D-18 during MWM test. All values are Mean±Standard Error of Mean (SEM, *n*=6). [a]*p*<0.05 compared to Control and [b]*p*<0.05 compared to Sham (Two-way ANOVA followed by Bonferroni *post-hoc* test for escape latency from D-14 to D-17 and for other parameters one-way ANOVA followed by Student-Newman-Keuls *post-hoc* test).

STZ Impairs Spatial Working Memory Formation in Y-Maze Test

Representative track plot of rat during Y-maze test was illustrated in Figure 3(A). Effect of STZ on change in spatial learning and memory in terms of percentage SAB of animals is depicted in Figure 3(B). STZ significantly decreased the percentage of SAB during Y-maze test ($F_{2,15}$ = 63.09) compared to other group rodents.

STZ Impairs Cholinergic Activity in Rat HIP, PFC and AMY

Figure 4 illustrated the effect of STZ on cholinergic function in discrete region of brain in terms of activity

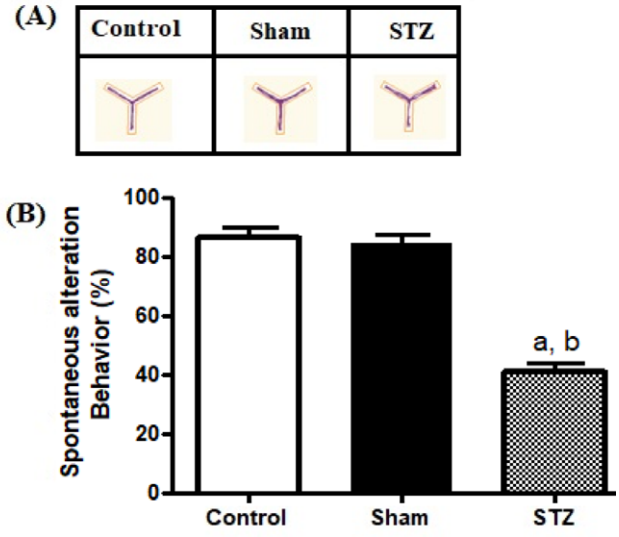

Figure 3: Representative tracking plot (A) of an animal from all groups on D-18 in Y-maze test paradigm; Effect of STZ on the percentage of spontaneous alteration behavior (SAB; B) of animals in Y-test paradigm. All values are Mean ± SEM (*n*=6). [a]*p*<0.05 compared to control and [b]*p*<0.05 compared to Sham (One-way ANOVA followed by Student-Newman-Keuls *post-hoc* test).

of ChAT (A), ACh level (B) and activity of AChE (C). STZ infusion significantly attenuated the activity of ChAT and the ACh level and increased the AChE activity in HIP [($F_{2,15}$ = 32.5), ($F_{2,15}$ = 40.90) and ($F_{2,15}$ = 27.30) respectively], PFC [($F_{2,15}$ = 90.0), ($F_{2,15}$ = 37.50) and ($F_{2,15}$ = 19.7) respectively] and AMY [($F_{2,15}$ = 122.7), ($F_{2,15}$ = 20.0) and ($F_{2,15}$ = 20.20) respectively] compared to other group animals.

STZ Increases the Level of Amyloid Beta in Rat Brain Regions

Effect of STZ on Aβ level is illustrated in Figure 5. STZ infusion significantly increased the level of Aβ in HIP ($F_{2,15}$ = 91.6), PFC ($F_{2,15}$ = 49.4), AMY ($F_{2,15}$ = 99.9) than other two group rats.

STZ Reduces the Expression Level of α7 nAChR in Discrete Rat Brain Regions

Figure 6 illustrated the effect of STZ on the expression level of α7 nAChR in selected rat brain regions. STZ infusion significantly attenuated the α7nAChR level in HIP ($F_{2,15}$ = 59.5), PFC ($F_{2,15}$ = 54.9) and AMY ($F_{2,15}$ = 76.8) compared to other two group rats.

STZ Attenuates Function and Integrity of Mitochondria in Rat Brain Regions

Figure 7 illustrated the effect of STZ on the function in the terms of the level of formazan produced in MTT assay (A) and integrity in terms of the fluorescence intensity of TMRM (B) of mitochondria in memory-sensitized rat brain regions. STZ infusion significantly decreased the function and integrity of mitochondria in HIP [($F_{2,15}$ = 11.0) and ($F_{2,15}$ = 62.2) respectively], PFC [($F_{2,15}$ = 10.5) and ($F_{2,15}$ = 56.7) respectively] and AMY [($F_{2,15}$ = 8.1) and ($F_{2,15}$ = 54.2) respectively] compared to other two group rodents.

STZ Decreases Mitochondrial Bioenergetics in Rat Brain Regions

Effect of STZ on the alteration in the level of O_2 utilization in different stages of Oxygraph in rat HIP (A), PFC (B) and AMY (C) is depicted in Figure 8. The effect of STZ on the mitochondrial RCR (A) and ADP/O (B) is illustrated in Figure 9. STZ infusion significantly decreased RCR and ADP/O in rat HIP [($F_{2,15}$ = 19.4) and ($F_{2,15}$ = 96.0) respectively], PFC [($F_{2,15}$ = 20.1) and ($F_{2,15}$ = 27.3) respectively] and AMY [($F_{2,15}$

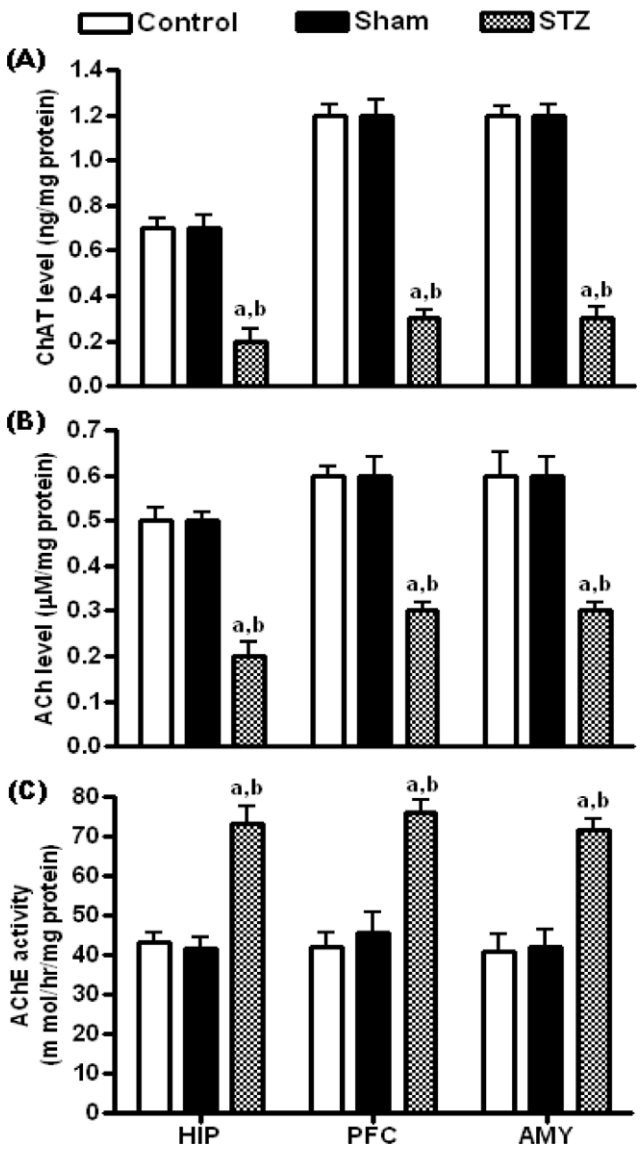

Figure 4: Effect of STZ on change in the level of choline acetyltransferase (ChAT; A) and acetylcholine (ACh; B) and activity of acetylcholinesterase (AChE; C) in the rat hippocampus (HIP), pre-frontal cortex (PFC) and amygdala (AMY). All values are Mean ± SEM (*n*=6). [a]*p*<0.05 compared to control and [b]*p*<0.05 compared to Sham (One-way ANOVA followed by Student-Newman-Keuls *post-hoc* test).

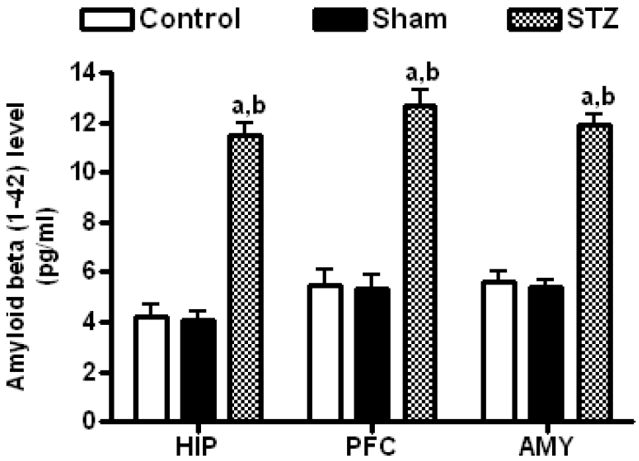

Figure 5: Effect of STZ on the level of amyloid beta (Aβ) in the rat HIP, PFC and AMY. All values are Mean ± SEM (*n*=6). [a]*p*<0.05 compared to Control and [b]*p*<0.05 compared to Sham (One-way ANOVA followed by Student-Newman-Keuls *post-hoc* test).

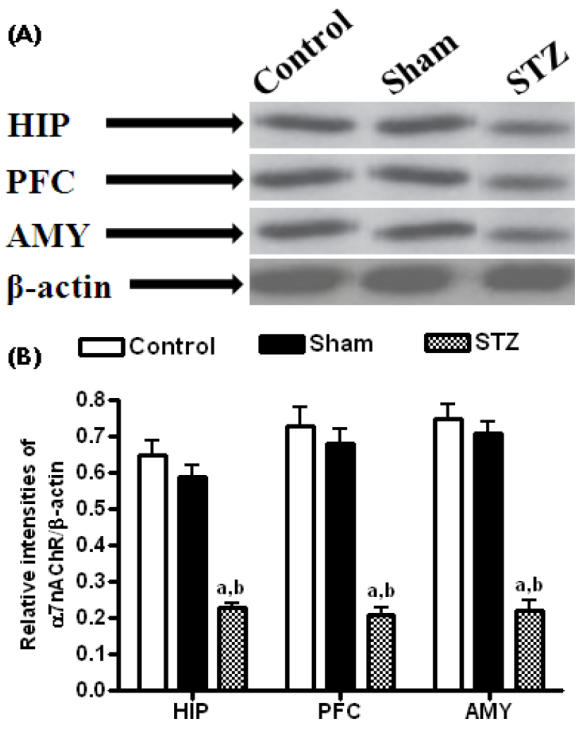

Figure 6: Effect of STZ on the level of expression of α7 nACh receptor in the rat HIP, PFC and AMY. All values are Mean ± SEM (*n*=6). [a]*p*<0.05 compared to control and [b]*p*<0.05 compared to Sham (One-way ANOVA followed by Student-Newman-Keuls Post-hoc test).

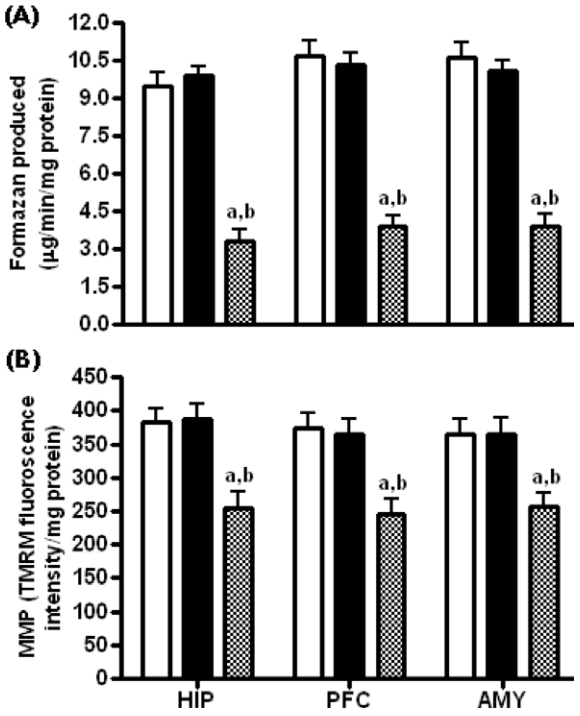

Figure 7: Effect of STZ on the mitochondrial function in terms of level of formazan produced in MTT assay (A) and integrity in terms of fluorescence intensity of TMRM (B) in the rat HIP, PFC and AMY. All values are Mean ± SEM (*n*=6). [a]*p*<0.05 compared to control and [b]*p*<0.05 compared to Sham (One-way ANOVA followed by Student-Newman-Keuls *post-hoc* test).

= 24.4) and ($F_{2,15}$ = 92.2) respectively] compared to other two group animals.

STZ Increases Markers of Oxidative and Nitrosative Stress in the Rat Brain Regions

Effect of STZ on mitochondrial level of NO and LPO and activity of CAT and SOD in rat HIP, PFC and AMY is illustrated in Table 1. STZ infusion significantly

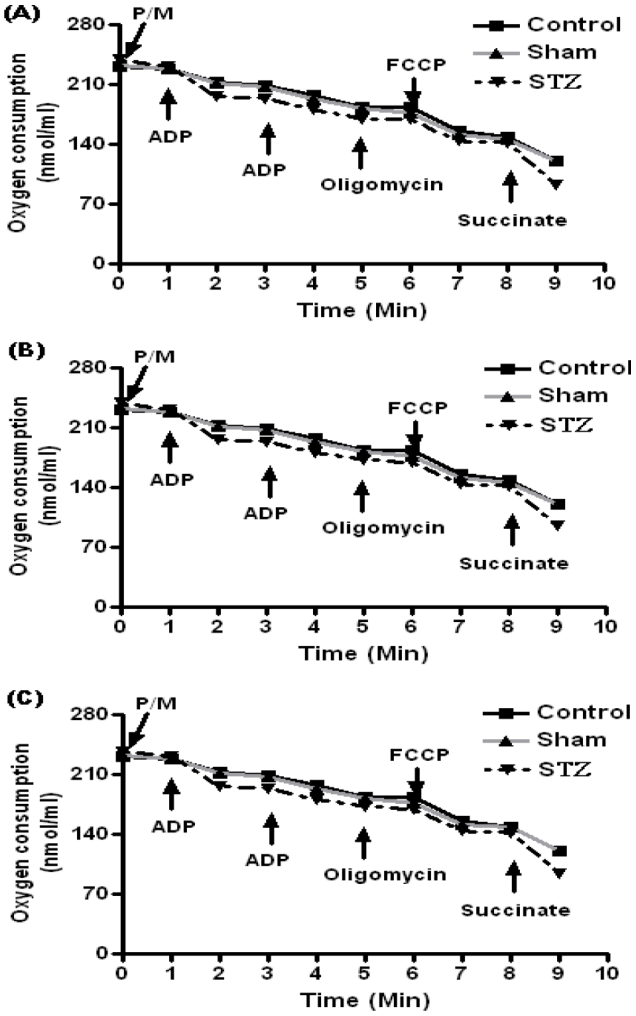

Figure 8: Representative Oxygraph of HIP (A), PFC (B) and AMY (C) of an animal in each group of the experimental protocol to show the level of oxygen consumption in different stages of mitochondrial respiration. Briefly, at first the initial rate of oxygen consumption (state 2 orV2) was recorded after addition of pyruvate plus malate (10 mM/5 mM). Further, the state 3 rate of oxygen consumption was recorded after addition of ADP (250 nmol). Moreover, a measurable state 4 rate of oxygen consumption (i.e., the rate after ADP phosphorylation) was monitored when a second pulse of ADP was added but the phosphorylative cycle was soon inhibited before its completion by adding oligomycin (1 µg). Subsequently, a measurable oligomycin oxygen consumption rate was recorded after FCCP (1 µM) was added to obtain a rate of oxygen consumption in the absence of coupled oxidative phosphorylation. Further, the oxygen consumption was recorded in presence of succinate (15 mM) and rotenone (2.2 mM) to observe the respiratory complex-II activity.

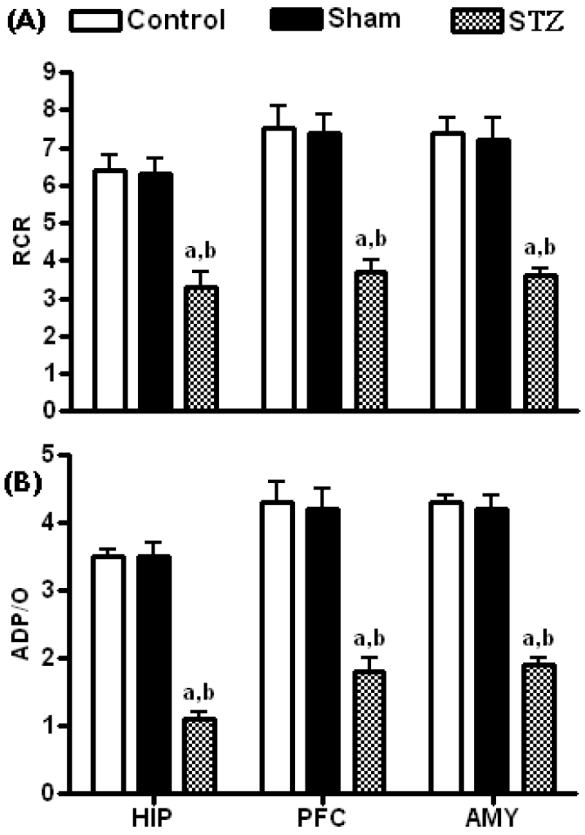

Figure 9: Effect of STZ on the mitochondrial respiratory control ratio (RCR; A) and ADP/O (B) in rat HIP, PFC and AMY. All values are Mean ± SEM (*n*=6). [a]*p*<0.05 compared to Control and [b]*p*<0.05 compared to Sham (One-way ANOVA followed by Student-Newman-Keuls *post-hoc* test).

increased the mitochondrial NO and LPO levels and decreased CAT and SOD activity in HIP [($F_{2,15}$ = 48.5), ($F_{2,15}$ = 50.0, ($F_{2,15}$ = 15.6) and ($F_{2,15}$ = 63.4) respectively], PFC [($F_{2,15}$ = 97.5), ($F_{2,15}$ = 55.5), ($F_{2,15}$ = 24.9) and ($F_{2,15}$ = 17.9) respectively] and AMY [($F_{2,15}$ = 26.0), ($F_{2,15}$ = 26.2), ($F_{2,15}$ = 18.4) and ($F_{2,15}$ = 38.5) respectively] compared to other two group rats.

DISCUSSION

We for the first time report a reduction in the α7 nAChR level in the brain regions of the STZ-challenged animals. Further, ICV injection of STZ impairs function, integrity and bioenergetics of mitochondria in the rat brain regions. Results also indicate a remarkable reduction in cholinergic function and elevation in the Aβ level in the above rat brain regions. Thus, there may be a relationship between the level of expression of alpha7 nAChR and function of mitochondria in the STZ-challenged brain areas of the rats.

The present study also suggests that ICV injection of STZ significantly reduced the learning and memory during MWM test paradigm and decreased the spatial

Table 1: Effect of STZ on mitochondrial level of NO and LPO and activity of SOD and CAT in rat HIP, PFC and AMY.

Tissue	Control	Sham	STZ
NO (μM MDA/mg protein)			
HIP	1.1 ± 0.03	1.2 ± 0.12	2.3 ± 0.11 [a,b]
PFC	1.3 ± 0.02	1.2 ± 0.06	2.5 ± 0.11 [a,b]
AMY	1.3 ± 0.03	1.3 ± 0.04	2.4 ± 0.21 [a,b]
LPO (nmol of MDA/mg of protein)			
HIP	0.6 ± 0.01	0.5 ± 0.03	0.9 ± 0.04 [a,b]
PFC	0.9 ± 0.02	1.0 ± 0.04	1.9 ± 0.12 [a,b]
AMY	0.9 ± 0.13	0.8 ± 0.13	1.8 ± 0.03 [a,b]
SOD (Units/min/mg of protein)			
HIP	0.5 ± 0.02	0.5 ± 0.03	0.2 ± 0.01 [a,b]
PFC	0.7 ± 0.07	0.8 ± 0.08	0.3 ± 0.02 [a,b]
AMY	0.8 ± 0.05	0.8 ± 0.06	0.3 ± 0.02 [a,b]
CAT (Units/min/mg of protein)			
HIP	2.1 ± 0.11	2.1 ± 0.13	1.3 ± 0.11 [a,b]
PFC	2.7 ± 0.03	2.6 ± 0.10	1.7 ± 0.16 [a,b]
AMY	2.7 ± 0.19	2.7 ± 0.14	1.6 ± 0.10 [a,b]

All values are mean ± SEM (n = 6). [a]p<0.05 compared to Control and [b]p<0.05 compared to Sham (one-way ANOVA followed by Student–Newman–Keuls test).

memory during Y-maze test as reported previously.[23] STZ treatment showed significant decrease in the learning ability of the rats in this protocol throughout the study (D-14 to D-17). Further, STZ exhibited significant decrease in the percentage of total distance travelled and time spent in the target quadrant in the probe session, indicating that STZ attenuated memory formation. Additionally, STZ significantly attenuated spatial memory in terms of percentage of SAB during Y-maze paradigm, suggesting the fact that STZ reduces the short-term memory formation. It is well established that the AD leads to decline in MWM task performance in rodents and MWM learning and memory status provide a good approach for screening of agents with anti-AD potential.[44] In addition to this, ICV injection of STZ also increased Aβ level in the above all three brain regions as appears in AD. Our findings show that the cholinergic dysfunction associated with alterations in mitochondrial bioenergetics and oxidative status could be considered as early events in pathophysiology of AD. ICV-STZ administration can induce various physiological, pathological and behavioral changes in animals including memory impairment, metabolic dysfunctions, oxidative stress and many more.[45] STZ has been shown to enhance AChE activity in cognitive deficits sensitized rodent brain.[46,47] In the present study, AChE activity was significantly elevated in STZ-challenged rat brain which is similar to that of Sonkusare *et al.*[48] ICV-STZ injection

in rats leads to progressive deficits in memory and learning and also develops various pathological aspects similar to AD.[25] In addition to increase in the AChE activity, ChAT performance also diminishes in STZ-challenged rodents. Thus, cholinergic dysfunction also leads to diminish the rate of ACh synthesis in memory-sensitized rat brain regions.[49,50] It is well reported that excessive reactive oxygen species (ROS) generation in neuronal cell also contribute in the phathogenesis of AD.[51,52] Mitochondria are the primary source of energy and produce about 90-95% cellular ATP by oxidative phosphylation. The mitochondrial ETC and NADPH oxidase are considered as the most important source for cellular ROS. Mitochondrial dysfunction leads to reduced ATP production and results into energy metabolic crises.[53,54] impaired energy metabolisms has been reported in AD-like brain. Increased oxidative stress derived from mitochondrial dysfunction facilitates the neurodegeneration.[45] Further, ICV-STZ injection altered bioenergetics and mitochondrial integrity that leads to the inhibition of ATP synthesis in memory-sensitized brain rat which is similar to pervious finding.[47] Thus, ICV-STZ administration induced oxidative stress dependent mitochondrial dysfunction and it may be postulated that energy impairment caused by STZ may be a potential source for this oxidative stress.

Loss or down regulation of the neuronal nAChRs is also reported in AD pathophysiology.[55] We also found that ICV-STZ administration altered the expression of α7 nAChR in memory-sensitized rat brain which led to persistent impairment in learning and memory and quantified in terms of decrease expression of α7 nAChR in above brain regions of rats. The nAChRs especially α7 nAChRs are well expressed in the neuron of brain and mainly in HIP, PFC and AMY. It has been well accepted that α7 nAChRs activation is a useful therapeutic approaches for management of AD because this receptor maintains the cholinergic phenotype.[25,56] Thus, it can be assumed that there may be a significant role of α7 nAChRs in the STZ-challenged memory-sensitized brain regions of cognitive impaired rats which is a matter of future experiments.

CONCLUSION

In conclusion, STZ treatment attenuates α7 nAChR expression and mitochondrial dysfunctions in all three memory-ensitized rat brain regions. Further, the cholinergic functions are reduced in STZ treated rat brain and level of Aβ is increased in all three brain regions of such animals. Therefore, results indicate that there could be a strong relationship between α7 nAChR expression and dysfunction in mitochondria in memory-sensitive areas of STZ-challenged animals. Thus, it can be speculated that α7 nAChR-dependent mitochondrial function could be an alternate target to enhance memory formation in AD.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

ABBREVIATIONS

AD: Alzheimer's disease; **nAChRs:** Nicotinic Acetylcholine Receptors; **Aβ:** Amyloid Beta; **ACh:** Acetylcholine; **ROS:** Reactive Oxygen Species; **ICV:** Intracerebroventricular; **STZ:** Streptozotocin; **aCSF:** Artificial Cerebrospinal Fluid; **IEAC:** Institutional Animal Ethics Committee; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments in Animals' **AChE:** Acetylcholinesterase; **ChAT:** Choline Acetyltransferase; **MWM:** Morris Water Maze; **SAB:** spontaneous alteration behavior; **HIP:** Hippocampus; **PFC:** Prefrontal Cortex; **AMY:** Amygdale; **ANOVA:** Analysis of Variance; **MTT:** 3-(4, 5-Dimethylthiazol-2-yl)-2, 5 Diphenyltetrazolium Bromide; **TMRM:** Tetramethyl Rhodamine Methylester; **MMP:** Mitochondrial Membrane Potential; **RCR:** Respiratory Control Ratio; **LPO:** Lipid Peroxidation; **MDA:** Malonaldialdehyde; **CAT:** Catalase; **SOD:** Super Oxide Dismutase.

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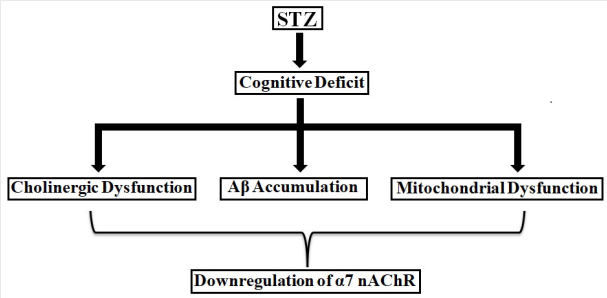
PICTORIAL ABSTRACT



SUMMARY

- Intracerebroventricular infusion of streptozotocin into the lateral ventricles caused a downregulation in the α7 nACh receptor in the memory-sensitive rat hippocampus, pre frontal cortex (PFC) and amygdale.
- Intracerebroventricular streptozotocin infusion caused cholinergic dysfunction and accumulation of amyloid beta in these brain regions.
- Intracerebroventricular streptozotocin infusion impaired mitochondrial function, integrity and bioenergetics in these brain regions.
- The α7 nACh receptor could be a potential alternative target in the management of sporadic type of Alzheimer's disease.

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