The hippocampal neuroinflammatory markers in intracerebroventricular streptozotocin injected rats are correlated with the memory impairments at different time points of post-injection.

Nicky Singh
University of Calcutta - Rajabazar Science College Campus
Rupsa Ghosh
University of Calcutta - Rajabazar Science College Campus
Debasish Bandopadhyay
University of Calcutta - Rajabazar Science College Campus
Anupam Bandyopadhay
Serampore College
Tusharkanti Ghosh (tusharkantighosh53@yahoo.in)
University of Calcutta - Rajabazar Science College Campus

Research Article

Keywords: Streptozotocin, WME, Neuroinflammation, TNFα, COX2, PGE2

Posted Date: December 7th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2256162/v1

Abstract

Intracerebroventricular (ICV) injection of streptozotocin (STZ) induces memory impairments and neuroinflammation in rats but the relation between neuroinflammation and memory impairments in ICV-STZ injected rats is not well understood. In the present study the memory impairments [working memory errors (WME) and reference memory errors RME)] in ICV-STZ injected rats were assessed with concomitant changes in the neuroinflammatory markers (TNF α, IL-1β, COX2, PGE2) in hippocampus at five different time points after ICV-STZ injection (3rd hour to 21st day post injection). Results showed that the WME and RME were increased gradually with time, and the neuroinflammatory markers were also gradually increased concomitantly after ICV STZ injection. These higher levels of the inflammatory markers are correlated with the memory impairments (WME and RME) and probably indicate a link between memory impairments and neuroinflammation.

Introduction

Several chemically induced animal models are used to investigate the pathogenesis of dementia in AD like ICV injection of colchicines (Sil et al. 2015), okadaic acid (Costa et al. 2012) and amyloid beta peptide (Gupta et al .2018). Intracerebroventricular (ICV) injection of streptozotocin (STZ) in rats/ mice is a well-established method to develop rat model of sporadic Alzheimer's disease (Nazem et al. 2015; Rai et al. 2013). Streptozotocin is a glucosamine nitrosurea compound isolated from *Streptomyces achromogenes*(Lewis and Barbiers 1959) and was initially used as an antibiotic (Vavra et al. 1960). STZ can influence the glucose entry into neurons, astrocytes and other cells in brain by binding with glucose transporter 2 (GLUT 2) on the cell membrane(Lenzen 2008).

The learning, memory and cognitive behaviors were studied in ICV STZ injected rats and mice by several investigators (Salkovic-Petrisic et al. 2006; Ishrat et al. 2009; Mehan et al. 2012; Halawany et al. 2017; Nakhate et al. 2018). The memory in Morris water maze escape task was impaired after 2 weeks of ICV STZ injection in rats at the dose of 1.5mg/kg body wt (Blokland and Jolles et al, 1994). The memory and learning were also impaired in ICV STZ injected rats after 3–4 weeks at the dose of 1mg/kg body wt (Salkovic-Petrisic et al. 2006) and 1.25 mg/kg body wt (Prickaerts et al. 2000).The memory parameters as measured in elevated plus maze and Morris water maze in ICV STZ injected rats were reported to be disrupted after 3–4 weeks at the dose of 3mg/kg body wt (Dhull et al. 2012) and 6mg/kg body wt (Rai et al. 2013). The memory deficit (measured by Morris water maze) induced by ICV STZ injection in rats at the dose of 1.85mg/kg body weight was found to be progressive which was identified at 2 week of injection and was maintained up to 12 weeks post treatment (Shoham et al. 2003).The working memory error and reference memory error were impaired in radial arm maze in ICV STZ injected rats at the dose of 3mg/kg body wt after 3 weeks of injection (Ghosh et al. 2020). The memory assessment in Y maze and Morris water maze indicated impaired memory in ICV STZ injected rats at the dose of 3mg/kg body wt after 18 days of injection. (Sharma et al. 2020).The changes of memory parameters in ICV STZ model of rats as observed by different investigators depend on the dose of STZ and also on the time point at which memory parameters were measured after ICV STZ injection in these animals (Ghosh et al. 2020).

The optimum dose of STZ for induction of memory impairments was investigated from the dose response curve of the memory parameters in ICV STZ rats and it was found to be 3mg/kg body wt (Ghosh et al. 2020: Homolok et al. 2020)).

The neuroinflammation in ICV injected rats were investigated by several investigators (Chen et al. 2020; Ghosh et al. 2020).The neuroinflamation as measured by immunohistochemical studies in ICV STZ injected rats after3 weeks of injection was detectable at low dose (1mg/kg body wt) and pronounced at higher dose (3 mg/kg body wt) (Kraska et al. 2012). The level of TNF α was increased in ICV STZ injected rat brain at the dose of 3mg/kg body weight at 2 weeks (Ahmed et al. 2013), 3weeks (Singh and Kumar 2016) and 4 weeks (Reeta et al. 2013). The increased levels of TNF α, IL 1β, reactive oxygen species and nitrite in hippocampus and cortex of rats were reported in ICV STZ injected rats at the dose of 6 mg/kg body weight after 3 weeks (Rai et al. 2013). The increased level of neuroinflammatory markers (TNF α, IL 1β, ROS and nitrite) in the hippocampus of ICV STZ injected rats was reported at the dose of 3mg/kg body weight after 3 weeks of injection(Ghosh et al. 2020). A prominent increase in TNF-α was also seen in ICV STZ injected mice brain after 21 days at the dose of (3mg/kg body weight (Sirwi et al, 2021).

The neuroinflammatory markers and memory impairments in ICV STZ injected rats have been extensively studied at various doses (0.5 /kg body wt to 6 mg/kg body wt) and different time points (2–12 weeks) but the relation between neuroinflammation and memory impairments in ICV-STZ injected rats is not clearly understood. The role of neuroinflammatory markers on memory impairments may be investigated, if the levels of inflammatory markers in hippocampus are measured with concomitant changes in memory parameters at the early and late time points after ICV STZ injection. Hence, the present study has been designed to investigate the relation between neuroinflammatory markers (TNFα, IL-1β, COX2, PGE2) in hippocampus and memory impairments[working memory errors (WME) and reference memory errors (RME)] in ICV-STZ injected rats at different time points after ICV-STZ injection.

Materials And Method

Animals

Adult male Wistar rats weighing 200-250 g were used for this study. The rats were housed individually in standard laboratory condition maintained at 26 – 28 ^{0} C and with 12 hour light dark cycle. All the rats had access tofood and water. The protocols used in this study were carried out with the approval from the institutional Animal Ethics Committee.

Experimental design

Five experiments were carried out for 5 different time points in this study. In each experiment memory parameters (working memory error, reference memory error) were measured in 18 rats in a radial arm maze (testing for 5 days), after initial habituation (5 days) and training (15 days). 18 rats were equally divided into 3 groups: control (C, without any ICV injection), sham operated [S, ICV injection of artificial CSF (aCSF)] and ICV STZ injected (STZ-IR, ICV injection of STZ at 3 mg/kg body wt). The rats of STZ-IR

and S groups were subjected to stereotaxic surgery for implantation of guide cannula (for short duration study: 3- hour, 6-hour, 24- hour) or ICV injection of STZ or aCSF (through microcannulafor long duration study :7-day and 21-day). The memory parameters were measured in a RAM after recovery from surgery [vide below for details in short and long duration study] at different time points of ICV injection of STZ/aCSF in 5 different experiments (i.e, 3- hour, 6-hour, 24- hour, 7- day and 21- day) and then the rats were sacrificed (under ether anesthesia followed by decapitation) for collection of brain. The inflammatory markers (TNFα, IL1β, COX2, PGE2) were measured in hippocampus of 6 rats/group.

Short duration study (3-hour, 6-hour and 24-hour experiments in 19-day study): After habituation in a RAM (5 days) and training (15 days), the study started with the testing of rats for the memory parameters for 5 consecutive days(1^{st} to 5^{th} day of the study). Guide cannulae were implanted into lateral ventricles of both sides of brain stereotaxically (vide below for details) on 6^{th} day of study. These rats were again tested for memory parameters from 14^{th} day to 18^{th} day of study (i.e, on 8^{th} and 12^{th}day of guide cannula implantation). STZ/aCSF was microinfused into lateral ventricles of conscious rats with the help of a infusion needle (passing through guide cannula) on 19^{th} day of study (13^{th} day after guide cannula implantation). WME and RME were measured at 3^{rd}, 6^{th} and 24^{th} hour after microinfusion of STZ/aCSF. The rats were sacrificed after measurement of memory parameters as described earlier and tissues were collected for measurement of inflammatory markers.

Long duration study (7- day and 21- day experiments in 13-day / 27-day study): Like the short duration study, this study started with the testing of rats for the memory parameters for 5 consecutive days(1^{st} to 5^{th} day of the study),after habituation in a RAM (5 days) and training (15 days) . STZ/aCSF was microinfused through microcnnula into lateral ventricles of rats stereotaxically (vide below for details) on 6^{th} day of study. After recovery of 5 days WME and RME were measured on 12^{th} and 13^{th} day in 13-day study (corresponding to 6^{th} and 7^{th} day after STZ/aCSF ICV injection), and on 12^{th}, 15^{th}, 18^{th} , 21^{st}, 24^{th} and 27^{th} day in 27-day study (corresponding to 6^{th}, 9^{th}, 12^{th}, 15^{th}, 18^{th} and 21^{st} day after STZ/aCSF ICV injection). The rats were sacrificed on 13^{th} day of study (i.e, 7^{th} day after STZ/aCSF ICV injection) or 27^{th} day of study (i.e, 21^{st} day after STZ/aCSF ICV injection) after measurement of memory parameters as described earlier and tissues were collected for measurement of inflammatory markers .

Intracerebroventricular streptozotocin injection

Short duration study (3-hour, 6-hour and 24-hour experiments)

Guide cannula implantation

Rats were anesthetized with Na- thiopentone (40 mg/kg body weight, i.p) and head was fixed in the stereotaxic apparatus (ST141 INCO, Ambala, India) following the method described by Sil et al. (2015). After shaving and cleaning of the head midline incision was given on the scalp and Ligocane HCl (local anesthetic, Neon Laboratories, Mumbai, India) was applied on the cut surface of the skin and muscles to minimize pain during surgery. Two burr holes were made on the skull with a dental drill at AP -0.6 mm

from bregma , L ±1.5 mm, following the atlas of Paxinos and Watson (1986). Guide cannulae (stainless steel tubing, 1.2 mm diameter, 1.5 cm long) were implanted stereotaxically into the lateral ventricles of both sides at 2.6 mm depth from the cortical surface. Two guide cannulae were fixed on the skull with dental acrylic and an anchoring stainless steel screw. After surgery the muscle and the skin were sutured separately and Neosporin powder was sprayed over the sutured area.

Microinfusion procedure

After 12 days of cannula implantation (7 days recovery and 5 days for testing WME and RME) freshly prepared streptozotocin (Sigma –Aldrich, USA) solution in artificial CSF (10µl containing 3mg/kg body wt for STZ IR) or artificial CSF (10µl for Sham operated rats) was microinfused slowly (1µl/min) into the lateral ventricles of two sides (5 µl in each side) of the conscious rat by an infusion needle (stainless steel tubing, 0.55 mm diameter) inserted through the guide cannula. The length of the infusion needle was taken in such a way that the tip of the infusion needle remained 2 mm below the tip of the guide cannula. The infusion needle was attached to a 10µl syringe (Hamilton, USA) through polyethylene tubing. The infusion needle was kept in place for 2-3 min after ICV injection.

Long duration study (7-day and 21-day experiments)

Rats were anesthetized, mounted on the stereotaxic apparatus, and two burr holes were made on the skull (AP: -0.6mm from bregma, L: ± 1.5 mm) following the method described in short duration study. A stainless steel micro-cannula (0.45 mm diameter) connected to a 10 µl Hamilton syringe (Hamilton, USA) with polyethylene tubing was inserted slowly into lateral ventricle at 2.8 mm depth from cortical surface. 10 µl of Streptozotocin solution in artificial CSF (containing 3mg/kg body wt of streptozotocin for STZ IR) or artificial CSF (10µl for Sham operated rats) was microinfused slowly into the lateral ventricles of two sides (5 µl in each side) following the method described earlier. The trephine hole was sealed with sterile bone wax after withdrawal of microcannula. Muscles and skin were sutured separately and Neosprin powder was sprayed on the cut surface as antiseptic measure.

Working memory errors (WME) and Reference memory errors (RME)

The WME and RME were measured in a 8- Arm Radial Arm maze (RAM) according to the modified protocol of Mizuno et al. (2000) and protocol used in previous study from this laboratory (Sil et al. 2017). It consisted of habituation session (5 days), training session (15 days) and testing session (1-5 days, preoperative control). WME and RME were again measured in post operative days as mentioned in design of the study. The behaviours were recorded and analysed using Any maze software (Steoling, USA).

Habituation session

The habituation session was performed for 5 days with the schedule of 3 trials per day in which the rat was placed in the central zone of the radial arm maze apparatus(wooden,18.5×3.5×10.5 in.) and allowed to move freely in all the 8 arms where the chocolate chips were spread all over the platform. After visiting

all the 8 arms the rat was removed. Alcoholic solution was used to clean the RAM after each trial. The rats were food deprived by 30% of their daily dietary intake.

Training session

The training session was carried out for 15 days with the schedule of 3 trials per day. The maximum duration of the trial was 5 minutes for each rat. During the training session, the rats were trained to visit only the 4 arms which were baited. In the initial few trails the proximal end of the arms were baited then gradually in next few trials it was baited in the middle and in the later trials it was baited in the distal end of the arm.

Test session

Trained rats were tested for 5 days with the schedule of 3 trials per day before the surgery. The maximum duration of the trial was 5 min for each rat or it ended as soon as the rat visits all the 4 baited arms. The following memory parameters were measured: Reference memory errors (entry into any non- baited arm) and Working memory errors (repeated entry into the baited arm).

Post-operative WME and RME:

WME and RME were again tested in short duration study from 8^{th} to 12^{th} day after implantation of guide cannula, and at 3^{rd} hour, 6^{th} hour and 24^{th} hour after ICV injection of STZ/aCSF (i.e, on 13^{th} /14^{th} day of guide cannula implantation). In long duration study WME and RME were measured and on 12^{th}, 15^{th}, 18^{th} , 21^{st}, 24^{th} and 27^{th} day in 21-day study (corresponding to 6^{th}, 9^{th}, 12^{th}, 15^{th}, 18^{th} and 21^{st} day after ICV STZ injection).

Estimation of inflammatory markers

The hypocampi of both sides were collected from the isolated brain (while placing it on an ice cold platform) and these were homogenized in ice cold 1X PBS (pH 7.4). The hippocampal homogenate was centrifuged at 5000 g (4^{o} C for 5 min) and the supernatant was carefully collected for estimation of the inflammatory markers by ELISA.

TNFα

100 µl of the hippocampal supernatant was taken from the stock for the estimation of TNFα by a commercial rat TNFα ELISA kit (CUSABIO, Wuhan Huamei Biotech Co. Ltd) following the protocol prescribed in the kit. The absorbance was measured at 450nm in 96 well plate reader. The hippocampal TNF-α level was expressed as pg/100mg protein in the sample

IL 1β

100 µl of the hippocampal supernatant was taken from the stock for the estimation of IL1β by a commercial rat IL1β ELISA kit (Ray Bio, Norcross, GA) following the protocol prescribed in the kit. The absorbance was measured at 450nm in 96 well plate reader. The hippocampal IL 1β level was expressed as pg/mg protein in the sample.

Estimation of COX2

50 µl of the hippocampal supernatant was taken from the stock for the estimation of COX2 by a commercial rat COX-2 ELISA kit (Qayee-Bio, China) following the protocol prescribed in the kit. The absorbance was measured at 450nm in 96 well plate reader. The hippocampal TNF-α level was expressed as pg/100mg protein in the sample

PGE2

50 µl of the hippocampal supernatant was taken from the stock for the estimation of PGE2 by a commercial rat PGE2 ELISA kit (Elabscience, USA) following the protocol prescribed in the kit. The absorbance was measured at 450nm in 96 well plate reader. The hippocampal TNF-α level was expressed as pg/100mg protein in the sample.

Protein Estimation

The Protein content in the brain homogenate was estimated following the method of Lowry et al. (1951). Bovine serum albumin (BSA) 1mg/ml was used as standard (Rai et al. 2013).

Statistical analysis

For the statistical analysis of the data, Statistical Package for Social Science Software (SPSS software, version 20.0.0) was employed. To compare the data among control, sham and STZ-IR, one way ANOVA was used followed by post-hoc Tukey – Kramer multiple comparision test. To study the correlation between the memory parameters and neuroinflammatory markers, bivariate Pearson correlation was used. Data were expressed as mean ± SE.

Results

Memory Parameters

Working memory errors (WME):

The working memory errors in STZ-IR could not be measured at the 3^{rd} hour and 6^{th} hour of ICV-STZ injection as the rats stopped movement when they were placed in radial arm maze. But the working memory errors were significantly increased at the 24^{th}hour ($p < 0.001$), 7^{th}day ($p < 0.001$) and 21^{st} day ($p < 0.001$) of ICV STZ injection compared to the control and sham operated rats(fig1). WME also showed progressive increase in the last 3 time duration studies (i.e, at 24^{th} hour, 7^{th} day and 21^{st} day) after ICV-

STZ injection (fig 3A). In 21 days study, RME was measured from 6th day to 21st day (i.e, 12th to 27th day of study) at the interval of 2 days and the results showed a gradual increase in WME($p < 0.001$) after ICV STZ injection (fig 1E).

Reference memory errors (RME):

The reference memory errors in STZ-IR could not be measured at the 3rd hour and 6th hour of ICV-STZ injection as the rats stopped movement when placed in radial arm maze. But the reference memory errors were significantly increased at the 24thhour ($p < 0.001$), 7thday ($p < 0.001$) and 21st day ($p < 0.001$) of ICV STZ injection compared to the control and sham operated rats (fig 2). RME also showed progressive increase in the last 3 time duration studies (i.e, at 24th hour, 7th day and 21st day) after ICV-STZ injection (fig 3B). In 21 days study, RME was measured from 6th day to 21st day (i.e, 12th to 27th day of study) at the interval of 2 days and the results showed a gradual increase in RME($p < 0.001$) after ICV STZ injection (fig 2E).

Hippocampal inflammatory markers

TNFα :

The hippocampal TNFα level was significantly increased ($p < 0.001$) at all the time points in STZ-IR compared to the corresponding control and sham operated rats (Fig 4A). It was also observed that the hippocampal TNFα level was significantly higher at 6th hour, 24th hour, 7th day and 21st day ($p < 0.001$) compared to the previous time point (Fig 4A).

IL-1β :

The IL-1β level in the hippocampus of STZ-IR showed significant increase at all the time points compared to the corresponding control and sham operated rats at 6th hour, 24th hour, 7th day and 21st day ($p < 0.001$), but not in 3rdhour (Fig 4B). The hippocampal IL-1β level was also significantly increased at 6th hour, 24th hour, 7th day and 21st day ($p < 0.001$) compared to the previous time point (Fig 4B).

COX-2 and PGE2 :

The hippocampal level of COX-2 in STZ-IR showed significant increase at 3rd hour and 6th hour ($p < 0.01$), and at 24th hour, 7th day and 21st day ($p < 0.001$) compared to the corresponding control and sham operated rats. A significant increase in the hippocampal level of COX-2 was observed at, 24th hour, 7th day and 21st day ($p < 0.001$) compared to the previous time point (Fig 4C

The hippocampal level of PGE 2 in STZ-IR showed significant increase at all the time points ($p < 0.001$) compared to the corresponding control and sham operated rats. A significant increase in the hippocampal level of PGE2 was observed at 24th hour, 7th day and 21st day ($p < 0.001$) compared to the previous time point (Fig 4D).

Correlation between memory parameters and neuroinflammatory markers

When WME or RME in 5 different time point studies were correlated with the concomitant levels of 4 neuroinflammatory markers it was found that the WME and RME were significantly correlated with the changing levels of the observed neuroinflammatory markers (Table 1).

Table: 1. Correlation between WME/RME and concomitant levels of hippocampal 4 neuroinflammatory markers (TNFα, IL1β, COX2 and PGE2).

		Hippocampal TNFα	Hippocampal IL1β	Hippocampal COX2	Hippocampal PGE2
WME	Pearson's r	0.921	0.892	0.881	0.858
	p - value	0.001	0.001	0.001	0.001
RME	Pearson's r	0.991	0.987	0.979	0.970
	p - value	0.001	0.001	0.001	0.001

Discussion

The results of the present study showed that all the neuroinflammatory markers were increased in hippocampus after the ICV injection of STZ at all the time points experiments and the increase of neuroinflammatory markers were significantly increased from the previous time points in each experiments. The increase of TNF α and IL-1β in hippocampus after ICV injection at long duration study (2–4 weeks) was reported by several investigators (Ahmed et al. 2013, Reeta et al. 2013, Ghosh et al. 2020, Sirwi et al. 2021). The present study showed the pattern of change of TNF-α, IL-1β as well as COX2 and PGE2 at different time points after ICV STZ injection. It appears from the results of the present study that the inflammation initiated by STZ in brain was increasing with time and the maximum level was noted in the 21- day experiment.

STZ can influence the glucose entry into neurons, astrocytes and other cells in brain by binding with glucose transporter 2 (GLUT 2) on the cell membrane (Lenzen 2008). It was also reported that STZ injection in monkey impaired the insulin/IGF signalling genes expression in hippocampus and frontal cortex (Lee et al. 2014). Thus brain insulin resistance could be induced after ICV injection of STZ by these mechanisms (Grieb 2016). The reduced activities of glycolytic key enzymes in the cerebral cortex and hippocampus results after ICV administration of STZ (Plaschke and Hoyer 1993) which further leads to the decline in the level of ATP and creatine phosphate in neurons and other cells of brain (Lannert and Hoyer 1998; Nitch and Hoyer 1991). It is reported that ICV STZ administration causes a fall in neuronal glucose level, activation of microglia and astrocytes, overactivation of NMDA receptor and mitochondrial dysfunction leading to decreased ATP level, excitotoxicity, free radical formation and release of pro inflammatory mediators.

In the present study hippocampal TNF α, COX2 and PGE2 levels were increased even at the 3rd hour after the ICV injection of STZ, but IL-1β was not significantly increased at that time point. Thereafter all the observed neuroinflammatory markers were increased in STZ-IR compared to the corresponding control/sham operated rats. The time dependent increase of COX2 and PGE2 in STZ-IR was very little in short duration study but these markers gained very high level at 21st day after ICV STZ injection indicating the important contribution of COX2/PGE2 in neuroinflamtion at the long term study (Fig. 4C,D). However, the significant increase in COX2 and PGE2 at short duration study may also participate in the inflammatory process. On the other hand TNFα and IL-1β were steeply increased with time which probably indicates the participation of these two cytokines in both the short and long duration study (Fig. 4A, B).

The memory and cognitive behavior were reported to be impaired in rats by several investigatorat 2–12 weeks after ICV STZ injection(Salkovic-Petrisic et al. 2006; Ishrat et al. 2009; Mehan et al. 2011; Halawany et al. 2017; Nakhate et al, 2018). In the present study the early and late events of memory impairment were investigated and it was found that the WME and RME were increased at 24th hour, 7th day and 21st day of ICV STZ injection. Moreover, these memory errors were increased gradually with time and a linear increase has been indicated from the results from these 3 time points experiments. The time dependent pattern of the increase in WME and RME at the last 3 points may be related with the concomitant gradual increase in the inflammatory markers in hippocampus. The increase of WME and RME in 21- day experiment from 6th day to 21st day after ICV-STZ injection (i,e 12th day to 27th day of study) also supports the increasing impairments of memory with time, and which may be linked with gradual higher level of neuroinflammatory markers.

WME and RME could not be determined at 3rd and 6th hour after ICV STZ injection probably due to initial shock of the neurons involved in the circuitry controlling memory by increased level of TNFα, COX2 and PGE2 in hippocampus. STZ may have the possibility of a direct effect on these neurons. The procedure of microinfusion probably did not influence the memory behavior as the sham operated rats showed testing level of WME/RME at 3rd hour and 6th hour of ICV aCSF injection. The neuroinflammatory markers were not changed in the sham operated rats at these time points, and also at the other time point experiments of the present study, and these observations also indicate the increased level of inflammatory marker in STZ- IR is linked with the changes inWME and RME in STZ-IR. The significant correlation between WME/RME and the each of the neuroinflammatory markers in this study supports the contention of a link between WME/RME and the increased level of the observed neuroinflammatory markers induced by STZ in brain.

Thus the present study shows that the memory (WME and RME) were affected within 3rd to 6th hour of ICV STZ injection and memory impairments were gradually increased with time. The observed neuroinflammatory markers in hippocampus (TNFα, IL1β, COX2 and PGE2) were also concomitantly increased after ICV STZ injection. These higher levels of the inflammatory markers are correlated with the memory impairments (WME and RME) and probably indicate a link between memory impairments and neuroinflammation.

Declarations

Acknowledgements: This study was funded by Department of Science and Technology (DST), CSRI, Govt. of India [Reference No.SR/CSRI/353/2016(G) dt.24.4.2017]. Ms.Pritha Gupta is acknowledged for her assistance in a part of experimental work.

Funding source: This research work was funded by Department of Science and Technology (DST), CSRI, Govt. of India [Reference No.SR/CSRI/353/2016(G) dt.24.4.2017]

Competing Interest: The authors have no relevant financial and non-financial interest to disclose

Author contributions:

Nicky Singh Performed the experimental work and data collection.

Rupsa Ghosh Supported in a part of the experimental work.

Tusharkanti Ghosh Study conception and design., preparation of manuscript and participation in experimental work

Debasish Bandopadhyay Discussion of the results

Anupam Bandyopadhay Discussion of the results

Data availability: Data set generated during the present study are not available publicly but are available from the the corresponding author on reasonable request.

Ethics approval: The study has been approved by the Institutional Animal Ethics Committee, Dept of Physiology, University of Calcutta. [IAEC- V/T/TKG-DB-02 (Nicky Singh) / 2019 Dated on 7.8.19]

Consent to participate: Not applicable

Consent to publish: Not applicable

References

1. Ahmed ME, Khan MM, Javed H, Vaibhav K, Khan A, Tabassum R, Ashafaq M, Islam F, Safhi MM, Islam F (2013) Amelioration of cognitive impairment and neurodegeneration by catechin hydrate in rat model of streptozotocin-induced experimental dementia of Alzheimer's type. NeurochemInt 62:492–501
2. Blokland A, Jolles J (1993) Spatial learning deficit and reduced hippocampal ChAT activity in rats after an ICV injection of streptozotocin. PharmacolBiochemBehav44(2):491-494. doi:10.1016/0091-3057(93)90497-h

3. Chen L, Feng P, Peng A, et al (2020) Protective effects of isoquercitrin on streptozotocin-induced neurotoxicity. *J* Cell Mol Med 24(18):10458-10467. doi:10.1111/jcmm.15658
4. Costa AP, Tramontina AC, Biasibetti R, et al (2012) Neuroglial alterations in rats submitted to the okadaic acid-induced model of dementia. Behav Brain Res 226(2):420-427. doi:10.1016/j.bbr.2011.09.035
5. Dhull DK, Jindal A, Dhull RK, Aggarwal S, Bhateja D, Padi SS (2012) Neuroprotective effect of cyclooxygenase inhibitors in ICV-STZ induced sporadic Alzheimer's disease in rats. J MolNeurosci 46(1):223-235. doi:10.1007/s12031-011-9583-6
6. Ghosh R, Sil S, Gupta P, Ghosh T (2020) Optimization of intracerebroventricularstreptozotocin dose for the induction of neuroinflammation and memory impairments in rats. Metab Brain Dis 35(8):1279-1286. doi:10.1007/s11011-020-00588-1
7. Grieb P (2016) IntracerebroventricularStreptozotocin Injections as a Model of Alzheimer's Disease: in Search of a Relevant Mechanism. MolNeurobiol53(3):1741-1752. doi:10.1007/s12035-015-9132-3
8. Gupta P, Sil S, Ghosh R, Ghosh A, Ghosh T (2018) Intracerebroventricular Aβ-Induced Neuroinflammation Alters Peripheral Immune Responses in Rats. J MolNeurosci 66(4):572-586. doi:10.1007/s12031-018-1189-9
9. Halawany AME, Sayed NSE, Abdallah HM, Dine RSE (2017) Protective effects of gingerol on streptozotocin-induced sporadic Alzheimer's disease: emphasis on inhibition of β-amyloid, COX-2, alpha-, beta - secretases and APH1a. Sci Rep. 7(1):2902 doi:10.1038/s41598-017-02961-0
10. Homolak J, Perhoc AB, Knezovic A, OsmanovicBarilar J, Salkovic-Petrisic M (2020)Additional methodological considerations regarding optimization of the dose of intracerebroventricularstreptozotocin A response to: "Optimization of intracerebroventricularstreptozotocin dose for the induction of neuroinflammation and memory impairments in rats" by Ghosh et al., Metab Brain Dis 2020 July 21. Metab Brain Dis36(1):97-102. doi:10.1007/s11011-020-00637-9
11. Ishrat T, Parveen K, Khan MM, et al. (2009) Selenium prevents cognitive decline and oxidative damage in rat model of streptozotocin-induced experimental dementia of Alzheimer's type. Brain Res 1281:117-127. doi:10.1016/j.brainres.2009.04.010
12. Kraska A, Santin MD, Dorieux O, et al (2012) In vivo cross-sectional characterization of cerebral alterations induced by intracerebroventricular administration of streptozotocin. PLoS One 7(9):e46196. doi:10.1371/journal.pone.0046196
13. Lannert H, Hoyer S (1998) Intracerebroventricular administration of streptozotocin causes long-term diminutions in learning and memory abilities and in cerebral energy metabolism in adult rats. BehavNeurosci. 112(5):1199-1208. doi:10.1037//0735-7044.112.5.1199
14. Lee Y, Kim YH, Park SJ, et al (2014) Insulin/IGF signaling-related gene expression in the brain of a sporadic Alzheimer's disease monkey model induced by intracerebroventricular injection of streptozotocin. J Alzheimers Dis. 38(2):251-267. doi:10.3233/JAD-130776

15. Lenzen S (2008) The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia51(2):216-226. doi:10.1007/s00125-007-0886-7
16. Lewis C, Barbiers AR (1959) Streptozotocin, a new antibiotic. In vitro and in vivo evaluation. AntibiotAnnu7:247-254.
17. Mehan S, Meena H, Sharma D, Sankhla R (2011) JNK: a stress-activated protein kinase therapeutic strategies and involvement in Alzheimer's and various neurodegenerative abnormalities. J MolNeurosci 43(3):376-390. doi:10.1007/s12031-010-9454-6
18. Mizuno M, Yamada K, Olariu A, Nawa H, Nabeshima T (2000) Involvement of brainderivedNeurotrophic factor in spatialmemory formation and maintenance in a radial arm maze test in rats. J Neurosci 20:7116–7121
19. Nakhate KT, Bharne AP, Verma VS, Aru DN, Kokare DM (2018) Plumbagin ameliorates memory dysfunction in streptozotocin induced Alzheimer's disease via activation of Nrf2/ARE pathway and inhibition of β-secretase. Biomed Pharmacother 101:379-390. doi:10.1016/j.biopha.2018.02.052
20. Nazem A, Sankowski R, Bacher M, Al-Abed Y (2015) Rodent models of neuroinflammation for Alzheimer's disease. J Neuroinflammation. 12:74. doi:10.1186/s12974-015-0291-y
21. Nitsch R, Hoyer S (1991) Local action of the diabetogenic drug, streptozotocin, on glucose and energy metabolism in rat brain cortex. NeurosciLett128(2):199-202. doi:10.1016/0304-3940(91)90260-z
22. Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. Academic, San Diego
23. Plaschke K, Hoyer S (1993) Action of the diabetogenic drug streptozotocin on glycolytic and glycogenolytic metabolism in adult rat brain cortex and hippocampus. Int J DevNeurosci 11(4):477-483. doi:10.1016/0736-5748(93)90021-5
24. Prickaerts J, De Vente J, Honig W, et al (2000) Nitric oxide synthase does not mediate neurotoxicity after an i.c.v. injection of streptozotocin in the rat. J Neural Transm (Vienna) 107(7):745-766. doi:10.1007/s007020070056
25. Rai S, Kamat PK, Nath C, Shukla R (2013) A study on neuroinflammation and NMDA receptor function in STZ (ICV) induced memory impaired rats. J Neuroimmunol 254(1-2):1-9. doi:10.1016/j.jneuroim.2012.08.008
26. Reeta KH, Singh D, Gupta YK (2017) Chronic treatment with taurine after intracerebroventricularstreptozotocin injection improves cognitive dysfunction in rats by modulating oxidative stress, cholinergic functions and neuroinflammation. NeurochemInt108:146-156. doi:10.1016/j.neuint.2017.03.006
27. Salkovic-Petrisic M, Knezovic A, Hoyer S, Riederer P (2013) What have we learned from the streptozotocin-induced animal model of sporadic Alzheimer’s disease, about the therapeutic strategies in Alzheimer’s research. J Neural Transm 120:233–252
28. Salkovic-Petrisic M, Tribl F, Schmidt M, Hoyer S, Riederer P (2006) Alzheimer-like changes in protein kinase B and glycogen synthase kinase-3 in rat frontal cortex and hippocampus after damage to the insulin signalling pathway. J Neurochem 96:1005–1015

29. Sharma Y, Garabadu D (2020) Intracerebroventricular streptozotocin administration impairs mitochondrial calcium homeostasis and bioenergetics in memory-sensitive rat brain regions. *Exp Brain Res* 238(10):2293-2306. doi:10.1007/s00221-020-05896-7
30. Shoham S, Bejar C, Kovalev E, Weinstock M (2003) Intracerebroventricular injection of streptozotocin causes neurotoxicity to myelin that contributes to spatial memory deficits in rats. ExpNeurol 184(2):1043-1052. doi:10.1016/j.expneurol.2003.08.015
31. Sil S, Ghosh R, Sanyal M, Guha D, Ghosh T (2015) A comparison of neurodegeneration linked with neuroinflammation in different brain areas of rats after intracerebroventricular colchicine injection. J Immunotoxicol13(2):181-190. doi:10.3109/1547691X.2015.1030804
32. Sil S, Ghosh T, Ghosh R, Gupta P (2017) Nitric oxide synthase inhibitor, aminoguanidine reduces intracerebroventricular colchicine induced neurodegeneration, memory impairments and changes of systemic immune responses in rats. J Neuroimmunol 303:51-61 doi:10.1016/j.jneuroim.2016.12.007
33. Singh A, Kumar A (2016) Comparative analysis of intrahippocampal amyloid beta (1–42) and it is intracerebroventricularstreptozotocin models of Alzheimer's Disease: Possible behavioral, biochemical, mitochondrial,cellular and histopathological evidences. J Alzheimers Dis Parkinsonism 6:208
34. Sirwi A, El Sayed NS, Abdallah HM, et al. (2021) UmuhengerinNeuroprotective Effects in Streptozotocin-Induced Alzheimer's Disease Mouse Model via Targeting Nrf2 and NF-Kβ Signaling Cascades. Antioxidants (Basel) 10(12):2011. doi:10.3390/antiox10122011
35. Vavra JJ, Deboer C, Dietz A, Hanka LJ, Sokolski WT (1959) Streptozotocin, a new antibacterial antibiotic. Antibiot Annu.7:230-235.

Figures

Fig1

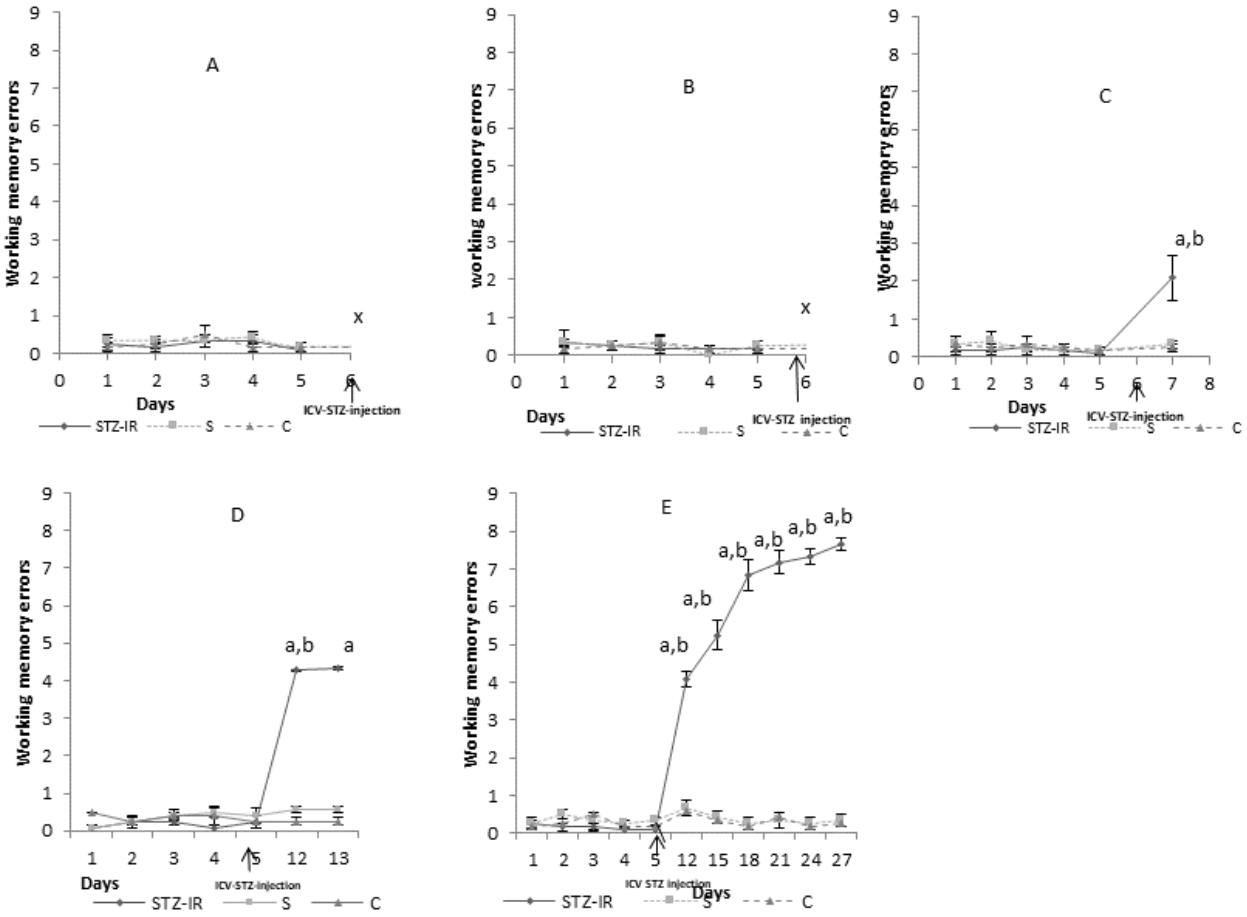

Figure 1

Working memory errors (WME) in control (C), sham operated rats (S) and ICV-STZ injected rats (STZ-IR) in different time duration studies. A: 3-hour, B: 6-hour, C: 24- hour, D: 7-day and E: 21-day experiments. [a]Significant ($p<0.001$) between STZ- IR and the corresponding time point of control/sham operated rats. [b]Significant ($p<0.001$) between a time point of STZ- IR compared to the previous time point of STZ-IR. X: The rats stopped movement after ICV-STZ injection at 3rd hour and 6th hour. Values are expressed in mean ± SEM (n= 6 rats)

Fig2

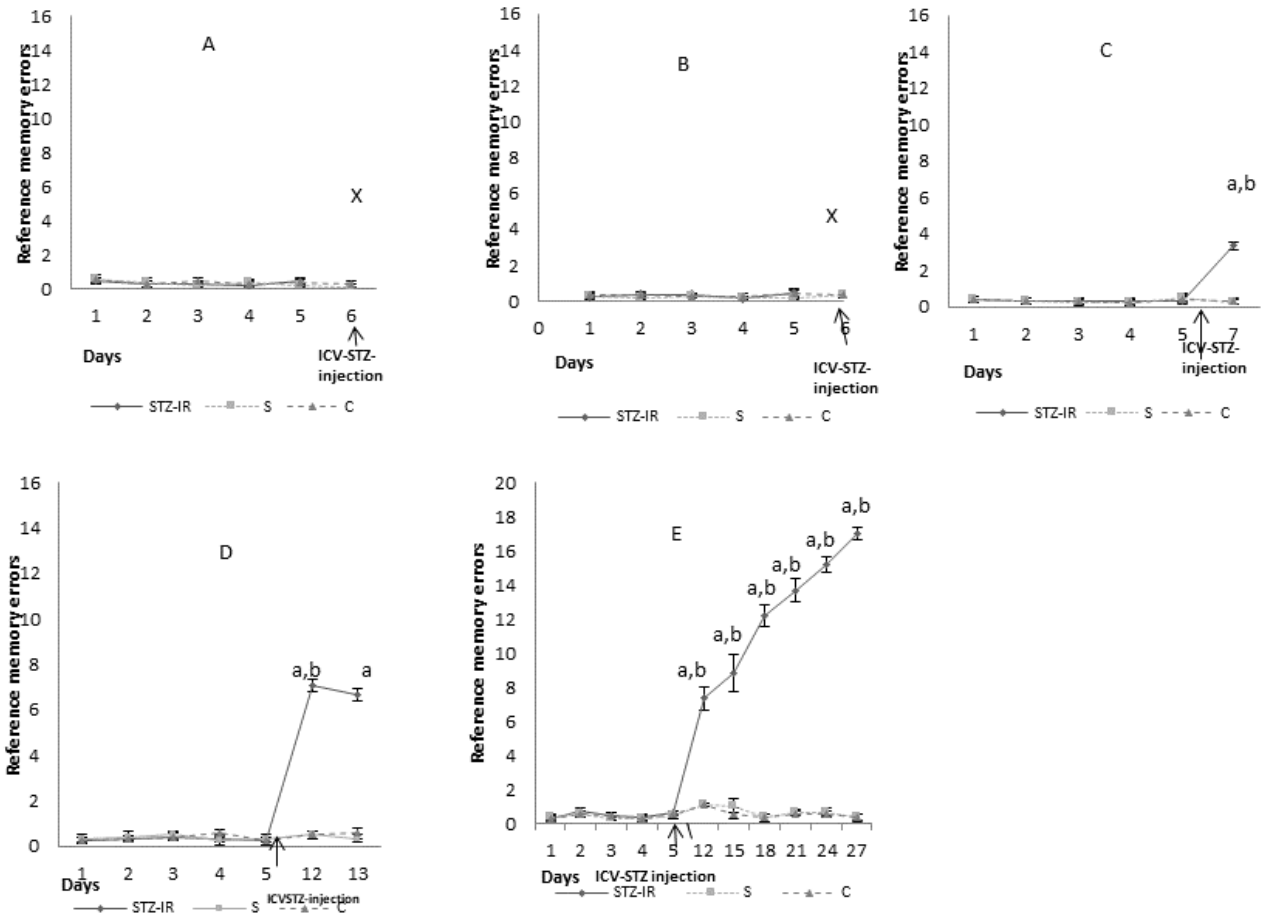

Figure 2

Reference memory errors (RME) in control (C), sham operated rats (S) and ICV-STZ injected rats (STZ-IR) in different time duration studies. A: 3 -hour, B: 6 - hour, C: 24-hour, D: 7-dayand E: 21-day. [a]Significant ($p<0.001$) between STZ IR and the corresponding time point of control/sham operated rats. [b]Significant ($p<0.001$) between a time point of STZ IR compared to the previous time point of STZ-IR. X: The rats stopped movement after ICV-STZ injection at 3rdhour and 6th hour. Values are expressed in mean ± SEM (n= 6 rats).

Fig3

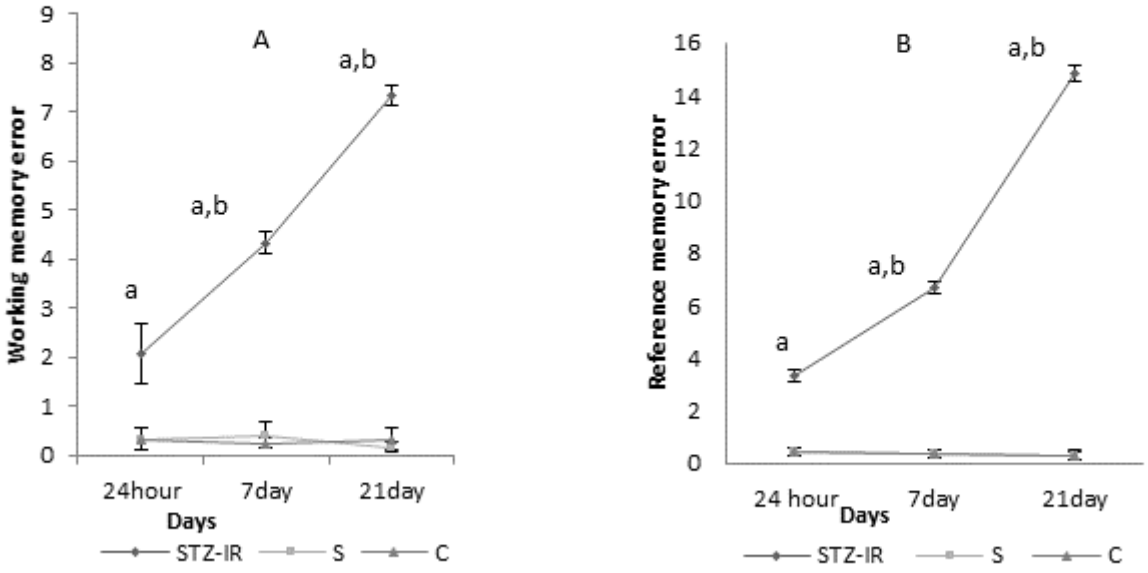

Figure 3

The time dependent changes of A: reference memory errors (RME) and B: working memory errors (WME) at 24th hour, 7th day and 21st day after ICV-STZ injection in STZ-IR rats along with WME/RME in control (C), sham operated rats (S). ^{a} Significant ($p<0.001$) between STZ- IR and the corresponding time point of control/sham operated rats. ^{b} Significant ($p<0.001$) between a time point of STZ-IR and the previous time point of STZ-IR.Values are expressed in mean ± SEM (n= 6 rats).

Fig4.

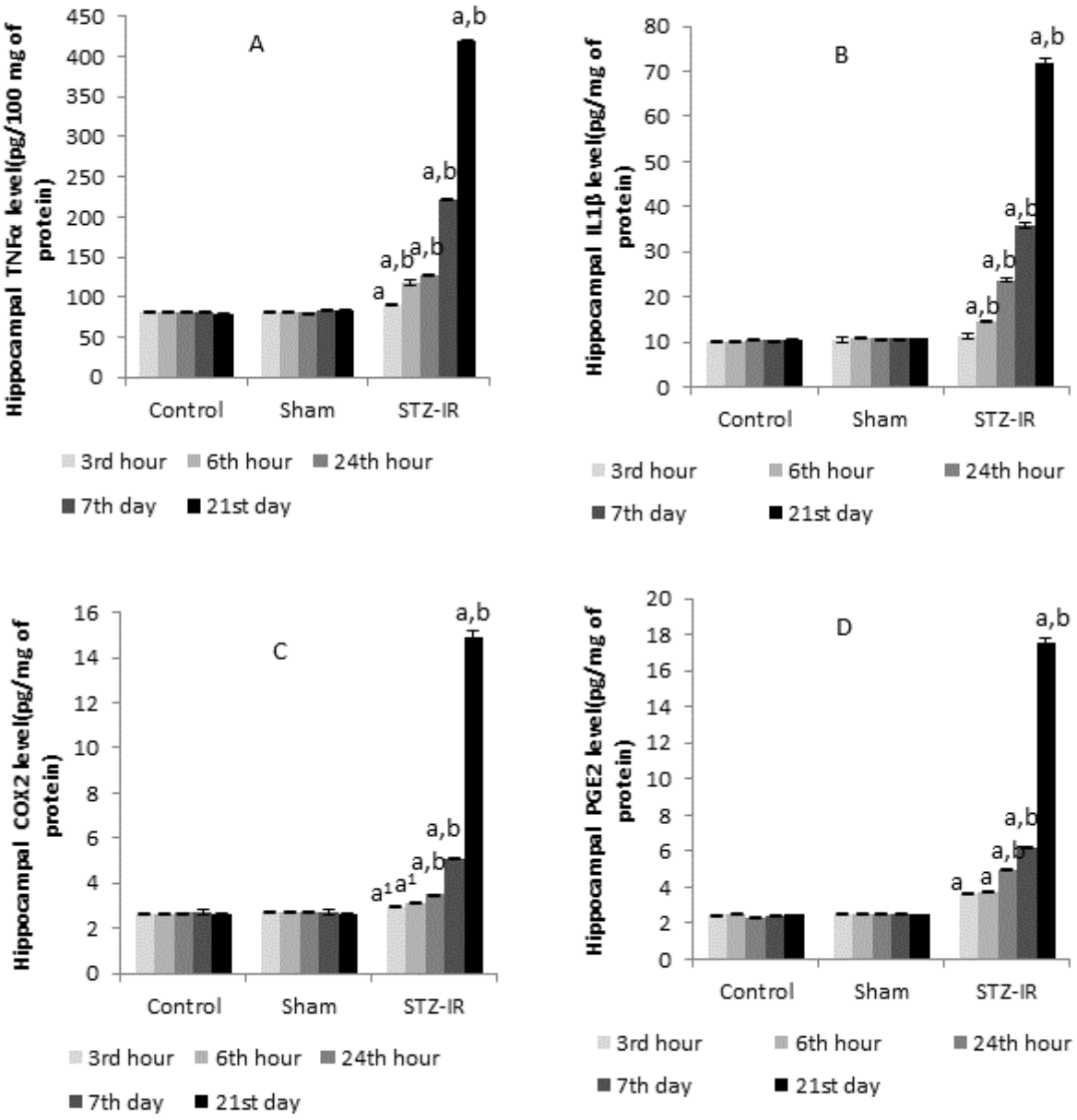

Figure 4

A: Inflammatory markers in hippocampus of different groups of rats in five time point experiments. A: Hippocampal TNFα, significantly higher (^{a} $p<0.001$) at all the time points compared to the corresponding control/ sham operated and significantly higher (^{b} $p<0.001$) compared to the preceding time point . B: Hippocampal IL-1β, significantly higher (^{a} $p<0.001$) at all the time points compared to the corresponding control/ sham operated and significantly higher (^{b} $p<0.001$) compared to the preceding time point. C: Hippocampal COX-2, significantly higher (^{a1} $p<0.01$ and ^{a} $p<0.001$) at different time points compared to the corresponding control/ sham operated and significantly higher (^{b} $p<0.001$) compared to the preceding time point.. D: Hippocampal PGE2, significantly higher (^{a} $p<0.001$) at all the time points compared to the

corresponding control/ sham operated and significantly higher (^{b} p<0.001) compared to the preceding time point.Values are expressed in mean ± SEM (n= 6 rats).