The effect of chronic stress and its preconditioning on spatial memory as well as hippocampal LRP1 and RAGE expression in a streptozotocin-induced rat model of Alzheimer’s disease

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Abstract
According to available evidence, prolonged or chronic exposure to stress is detrimental to various brain structures, including the hippocampus. The current study examined the expression of two critical blood–brain barrier receptors required for amyloid-beta clearance to understand better the mechanism by which chronic stress impairs learning and memory in patients with Alzheimer’s disease (AD). Rats were randomly assigned to one of two groups in this study: experiment 1 and experiment 2. Each main group was then divided into four subgroups. Rats were bilaterally injected with streptozotocin (STZ, 3 mg/kg, twice) using the intracerebroventricular (ICV) technique to induce the Alzheimer’s model. Additionally, they were subjected to foot shock (1 mA, 1 Hz) for 10 s every 60 s (1 h/day) for ten consecutive days prior to and following STZ injection. The Morris Water Maze (MWM) test was used to assess spatial learning and memory. Real-time PCR was used to determine Low-density lipoprotein receptor-related protein-1 (LRP1) and receptor for advanced glycation end-products (RAGE) mRNA levels in the hippocampus. Moreover, the animals’ body weights were determined as physiological parameters in all groups. The results indicated that 10-day chronic electric foot shock stress reduced body weight, impaired spatial learning and memory, decreased hippocampal LRP1 mRNA expression, and increased hippocampal RAGE mRNA expression in a rat AD model. It can be concluded that chronic stress in conjunction with AD alters the expression of LRP1 and RAGE in the hippocampus. The findings pave the way for scientists to develop novel treatment strategies for AD.

Keywords Alzheimer’s disease · Chronic stress · LRP1 · RAGE · Spatial learning and memory · Hippocampus

Introduction
Globally, the prevalence of age-related diseases has risen to become a significant public health concern. These diseases are well-known for causing progressive and permanent neuron loss, which results in dementia. Alzheimer’s disease (AD) is one of these diseases associated with aging (Mahdi et al. 2019; Yiannopoulou and Papageorgiou 2020). The disease is a progressive neurodegenerative illness characterized by multiple cognitive impairments and memory loss caused by intracellular tau aggregates and extracellular amyloid-beta (Aβ) accumulation (DeTure and Dickson 2019; Masters et al. 2015). The occurrence and prevalence of AD and other dementias have increased significantly over the last decade (Laurent et al. 2012). Thus, discovering cures or successful long-term treatment for AD is an urgent concern for healthcare.

Multiple approaches, including chemicals, have been used to induce AD-like symptoms in rats to evaluate various therapeutic agents for a range of cognitive dysfunctions. Streptozotocin (STZ) is one of the chemicals being studied to understand better the disease’s etiology (Mahdi et al. 2019; More et al. 2016). A link between diabetes and dementia was found at the turn of the previous century. According to previous research, diabetes and AD may
share a common pathophysiological mechanism (De la Monte and Wands 2008; Jia et al. 2017; Lester-Coll et al. 2006). STZ-induced experimental brain diabetes shares numerous similarities with AD (De la Monte and Wands 2008; Lester-Coll et al. 2006). Intracerebroventricular (ICV) injection of STZ is an important animal model of chronic brain dysfunction, characterized by progressive deficits in learning, memory, and cognition, an increase in Aβ−42, and a persistent and continuous cerebral energy deficit (Chen et al. 2013; Lester-Coll et al. 2006; Mahdi et al. 2019).

According to previous research, premature progressive AD may be caused by factors other than genetics and senescence (Machado et al. 2014). As per recent publications, environmental parameters such as chronic stress may contribute to the acceleration of AD pathogenicity. It has been reported that electric foot shock is used in animal models of depression, anxiety, and post-traumatic stress disorder (PTSD) as a validated model (Bali and Jaggi 2015a). Additionally, it is the most frequently used type of trauma in preclinical studies of PTSD (Bali and Jaggi 2015a, b). Increased exposure to environmental or psychological stressors is associated with the onset of AD, with these stressors playing a critical role in the disease’s progression (Cuadrado-Tejedor et al. 2012; Srivareerat et al. 2009). While numerous studies have established that chronic stress can impair memory in various animal models of AD (Machado et al. 2014), the role of stress in the pathogenesis of the ICV-STZ-induced sporadic type of AD remains unknown. Moreover, a complete understanding of the precise mechanism of action has not been attained.

The blood–brain barrier (BBB) is a diffusion barrier necessary for the proper function of the central nervous system (CNS). Along with the BBB, several receptors and transporters exist that enable molecules to pass through substrate-specific transport systems critical for AD pathogenesis (Govindpani et al. 2019; Weiss et al. 2009). For example, Low-density lipoprotein receptor-related protein-1 (LRP1) expression is moderate in the normal BBB, whereas receptor for advanced glycation end-products (RAGE) expression is minimal (Deane 2012; Sagare et al. 2012; Storck et al. 2016). Recent studies indicated that increased expression of RAGE and decreased expression of LRP1 were associated with various pathological states, including AD (Khodadadi et al. 2018; Miller et al. 2008; Montagne et al. 2017). Furthermore, it has been demonstrated that chronic or overwhelming stress has a detrimental effect on the brain, including the BBB (Kempuraj et al. 2019; Sántha et al. 2016; Urayama and Banks 2006). While research on the acute and chronic effects of stress on BBB dysfunction has been conducted, specific molecular and ultrastructural alterations have received insufficient attention. As a result, these changes are poorly understood.

By examining the changes in LRP1 and RAGE receptor expression in the hippocampus, we can better understand the mechanisms underlying neurological disorders such as stress and AD, enabling the development of disease-specific and efficacious therapeutic alternatives. The current study examined the effect of AD and chronic stress on hippocampal LRP1 and RAGE expression through two distinct study designs.

Methods

Animals

Male Sprague–Dawley rats weighing 200–250 g and aged approximately 12 weeks were obtained from the Laboratory Animal Center of Shiraz University of Medical Sciences (SUMS). Then, the animals were maintained under the standard condition of a 12 to 12 h light–dark cycle at 25 ± 2°C, and water and food were provided ad libitum. The experimental protocols were approved by the ethics committee of SUMS, and the animal care was according to the NIH Guide for the care and use of laboratory animals.

Experimental design

Two distinct sets of experiments were conducted to determine the effect of chronic stress on the AD rat model induced by STZ. The design of the study is represented in Fig. 1.

Experiment 1 (Exp 1) was conducted to determine the effect of stress on body weight, spatial learning and memory, and hippocampal LRP1 and RAGE expression prior to bilateral ICV-STZ infusion. Four groups of rats were used (7 rats per group): sham (rats received ICV normal saline), STZ (rats received ICV-STZ), stress (10 days of stress prior to ICV normal saline), and stress+STZ (10 days of stress prior to ICV-STZ). The animals were weighed on the first and the last day of the experiment, and final and initial body weight were recorded (Radahmadi et al. 2013). On days 11 and 13, rats received bilateral ICV injections of STZ (3 mg/kg). Additionally, the Morris Water Maze (MWM) test was performed on days 24–27 of the study. On day 28, the animals were sacrificed, and real-time PCR was used to assess hippocampal LRP1 and RAGE expression.

Experiment 2 (Exp 2) was conducted to determine the effect of stress on body weight, spatial learning and memory, and hippocampal LRP1 and RAGE expression after the bilateral ICV-STZ infusion. Four groups of rats were used (7 rats per group): sham (rats received ICV normal saline), STZ (rats received ICV-STZ), stress (10 days of stress after ICV injection of normal saline), and STZ+stress (10 days of stress after ICV-STZ). The animals were weighed on the
first and the last day of the experiment, and final and initial body weight were recorded. On days 1 and 3, rats received bilateral ICV injections of STZ (3 mg/kg). Additionally, the MWM test was performed on days 14–17 of the study. On day 18, the animals were sacrificed, and real-time PCR was used to assess hippocampal LRP1 and RAGE expression.

**Surgical procedure**

Rats were anesthetized on the operation date with an intraperitoneal injection of a ketamine (100 mg/kg) and xylazine (10 mg/kg) mixture. After mounting the animals into a stereotaxic frame, bilateral implantation of a stainless-steel guide cannula (22 gauge) was done into the lateral ventricle (AP-0.8, ML ± 1.5, DV-3.5) based on the paxinos brain atlas. Stainless screws and acrylic cement anchor these cannulas to the skull. In line with the literature, the rats receiving STZ (Sigma-Aldrich, Catalog No S0130 SIGMA) were subjected to two doses of 3 mg/kg on the first and third day (2.5 μl per lateral ventricle; in total 6 mg/kg). The sham group underwent similar surgical procedures, but their injections were administered with an equivalent amount of saline instead of STZ (Negintaji et al. 2015).

**Stress protocol**

A communication box was employed to deliver electrical foot shock stress. Transparent plastic sheets split this
instrument into nine divisions. The rats were subjected to psychophysical stress through an electrical foot-shock (1 mA, 1 Hz) at 10-s intervals every 60 s (1 h/day) for ten consecutive days through a stainless-steel grid. In contrast, control animals were not subjected to any stressors in their cages during the experiment. The control animals were then placed into the experimental room and handled the same way as the stress group, except receiving foot shock (Hormoz et al. 2018).

Evaluation of spatial learning and memory

The MWM test was used to assess spatial learning and memory. The apparatus consisted of a black round pool with a diameter of 140 cm and a height of 70 cm, filled to a depth of 25 cm with 20 °C water. The maze was divided into four equivalent quarters, with release points projected as N, E, S, and W at each quarter. A concealed round platform (11 cm in diameter) was located in the center of the southwestern quarter and submerged in 1.5 cm underneath the water surface. Additional maze visual signals were placed at different places around the maze (i.e., a computer, a door, a window, bookshelves, and posters). A charged coupled device (CCD) camera was fixed overhead the center of the maze in such a way to record animal activities and to send them to the computer. A computerized system (Noldus EthoVision, v13, Noldus Company, The Netherlands) was used to record the animal’s swimming route automatically. The procedure consisted of training sessions for four days. An unobservable platform was submerged almost 1.5 cm beneath the water surface during the primary three successive days. A block session comprised four trials with four different beginning locations. During every trial, the animal was allowed to discover the invisible platform. The rats were allowed to stay in the mounted platform for 20 s until the subsequent trial. The invisible platform was taken out, and the retention testing (probe trial) was implemented on the 4th day. Following the probe trial, an observable platform was positioned in another location to examine the rats’ motivation, visual ability, and sensory-motor coordination (Zarifkar et al. 2018). During days 1–3 of training, the swimming speed, escape latency to the hidden platform, frequency of entry into the target zone, and time spent in the target quadrant during the spatial probe test were measured.

Tissue collection and Real-time PCR analysis

After completion of the behavioral assessment, the animals were sacrificed. The hippocampus was quickly isolated on ice, was transferred to liquid nitrogen, and then stored in -72°C until the molecular analysis. According to the manufacturer’s protocol, the total RNA of hippocampus tissue (100 mg in weight) was extracted using RNA Sol reagent (Alpha Bio®, Cat. No: RSL 0050). RNA Sol isolation reagent is a mixture of Guanidium and phenol, which effectively dissolves DNA, RNA, and protein on homogenization or lysis of tissue samples. Then, cDNA was synthesized using a cDNA synthesis kit (EURx, Cat. No: E0801-01) according to the manufacturer’s instructions. An average of 500 ng/μl of the total RNA was used to synthesize copy DNA (cDNA) using Oligo (dT) 20 and random hexamer primers. The cDNA product was used in the RT-qPCR after the synthesis. All real-time PCR reactions were performed using SYBR Green real-time PCR kit (EURx, Cat. No: E0402-01). The real-time PCR conditions were as follows: initial activation at 95 °C for 1 min, 40 thermal cycles of denaturation at 95 °C for 10 s, primer annealing at 60 °C for 20 s, and DNA polymerase extension at 72 °C for 30 s. Then, the melting curve analysis was 95 °C for 15 s, and 60 °C for 1 min until 95 °C for 15 s. The PCR reaction included 5 μl master mix PCR, one μl forward and reverse Primers (Stoke 1 pm), 1.9 μl water 0.1μ Rox dye and 1 μl sample cDNA to a final volume of 10 μl. All qPCR reactions were performed in a StepOnePlus Real-Time PCR apparatus (Applied Biosystems), and gene expression levels were analyzed via the ΔΔCt method. Gene expression levels of the housekeeping gene, Gapdh, were used in each reaction to normalize the values determined. The specific primer sequences for RAGE were forward 5-CTGCCACCTTGGA TGGGAAAC-3 and reverse 5-CTGTCCCTGTATGCGTA TGA-3; for LR1 they were forward 5-CACATGGATGC CCCTAAAAC-3 and reverse 5-CTGGGCTTTTACTCTG TGGAC-3. For standardization, Gapdh was used as a reference gene, and the sequence of its primers is as follows: forward 5-GAGCAAGAGAGGAGGAGGA-3 and reverse 5-AGGGCCCTTCACT-AGGAC-3. Primer sequences were purchased from Pishgam co. (Iran) (Khodadadi et al. 2018). All data are represented as fold change versus sham and presented as mean ± SEM.

Statistical analysis

The results were indicated as the mean ± SEM. Analyses were done by using SPSS software version 22.0. The one-way ANCOVA (analysis of covariance) was used to determine whether there were statistically significant differences in body weight within groups in which initial weight was considered as covariate. Then, one-way ANOVA was used to determine differences in body weight between groups. The data from three days of spatial learning experiments were analyzed using repeated measures, and one-way ANOVA was used to compare how rats behaved on different training days. The remaining data were analyzed using a one-way ANOVA. Moreover, intragroup comparisons were made using Tukey’s post hoc test. In all the assessments, P < 0.05 was regarded as statistically significant.
Results

Experiment 1

Body weight difference

Table 1 indicates that there was no significant difference in body weight between the sham group and the stress group. On the other hand, this index dramatically decreased in the STZ and stress + STZ groups compared to the sham group (P < 0.01).

Spatial learning and memory

The performance of the Exp1 in the MWM test is indicated in Fig. 2. Figure 2A depicts the animals’ spatial learning abilities after three training days. The groups had a significant difference in escape latency (F (3, 24) = 31.12, P < 0.01). The mean escape latency in STZ groups (with or without stress) was substantially more prolonged than in sham and stress groups (P < 0.05). The mean escape latency of groups on each training day was analyzed using one-way ANOVA to compare how the animals behaved on different training days. According to the findings, there was a significant difference between groups on all days (Day 1: F(3, 24) = 20.48, P < 0.01). The results revealed no significant differences between groups (F(3, 24) = 0.01, P = 0.99). The data imply that stress and STZ did not affect the motivation or sensori-motor coordination of the animals.

Changes in LRP1 and RAGE mRNA

LRP1 and RAGE mRNA expression were analyzed in the hippocampus of rats by real-time PCR. The expression levels of LRP1 and RAGE in Exp1 are indicated in Fig. 3. Figure 3A shows the mRNA levels of LRP1 in the hippocampus of rats. There was a significant difference between groups (F(3, 8) = 356.7, P < 0.0001). The result showed that the mRNA levels of LRP1 were decreased in stress, STZ, and stress + STZ groups compared with the sham group (P < 0.05). Interestingly, this reduction was severe when animals received stress prior to STZ. Figure 3B shows the mRNA levels of RAGE in the hippocampus of rats. There was a significant difference between groups (F(3, 8) = 58.61, P < 0.001). On the other hand, although stress, STZ, or stress + STZ increased the mRNA levels of hippocampal RAGE, this enhancement was much more significant in the stress + STZ group (P < 0.05).

Experiment 2

Body weight difference

Table 2 represents that there was no significant difference in body weight between the sham group and the stress group. However, the body weight considerably reduced in the STZ and stress + STZ groups compared to the sham group (P < 0.01).

Spatial learning and memory

Figure 4 also shows the performance of the Exp2 in the MWM test. Figure 4A shows animals’ spatial learning ability during three consecutive training days. There was a significant difference in escape latency between the groups (F(3, 24) = 20.48, P < 0.01). The results showed that the mean escape latency in STZ groups (with or without stress) is significantly greater than in sham and stress groups (P < 0.05). The mean escape latency of groups on each training day was analyzed using one-way ANOVA to compare how the animals behaved on different training days. According to the findings, there was a significant difference between groups on all days (Day 1: F(3,
Results revealed that the escape latency of rats in the STZ groups (with or without stress) was significantly greater than that of rats in the sham and stress groups on all days \( (P < 0.05) \). However, on the first training day, rats in the stress group had a significantly longer escape latency than rats in the sham group \( (P < 0.05) \). Figure 4B shows the frequency of animals’ entrance into the platform area and its proximity. There was a significant difference between groups \( (F(3, 24) = 6.8, P < 0.01) \). The results showed that the frequency of entry into the platform area and its proximity STZ + stress and STZ groups is significantly different from the sham and stress groups \( (P < 0.05) \). Figure 4C shows the time spent in the platform area and its proximity. There was a significant difference between groups \( (F(3, 24) = 51.3, P < 0.01) \). The result showed that the time spent in the platform area and its proximity in stress and STZ groups (with or without stress) is significantly different from sham and stress groups \( (P < 0.05) \). Figure 4D shows the mean swimming velocity during days of training. There were no significant differences observed between groups \( (F(3, 24) = 1.67, P = 0.2) \), which means that the animal’s performance was not affected by the swimming speed. Figure 4E shows the escape latency to reach the visible platform. There were no significant differences between groups \( (F(3, 24) = 0.04, P = 0.99) \). This finding suggests that stress and STZ did not affect animals’ motivation or sensorimotor coordination.
Changes in LRP1 and RAGE mRNA

The expression levels of LRP1 and RAGE in the Exp2 are indicated in Fig. 5. Figure 5A shows the mRNA levels of LRP1 in the hippocampus of rats. There was a significant difference between groups (F(3, 8) = 255.9, P < 0.01). The results showed that the mRNA levels of LRP1 were decreased in the stress, STZ, and STZ + stress groups compared with the sham group (P < 0.05). Interestingly, this reduction was much less in the STZ + stress group. Figure 5B shows the mRNA levels of RAGE in the hippocampus of rats. There was a significant difference between groups (F(3, 8) = 48.99, P < 0.001). The result showed that the hippocampal RAGE mRNA levels were significantly upregulated in the STZ and stress groups compared to the sham group (P < 0.05). In addition, the mRNA levels of RAGE were increased in STZ + stress groups compared with other groups (P < 0.05).

Discussion

The present research evaluated the impact of chronic electric foot shock stress (before (Exp1) and after (Exp2) bilateral ICV-STZ injection) on body weight, spatial learning, memory, and hippocampal LRP1 and RAGE expression in a rat model of AD. According to the outputs, exposure to 10-day chronic electric foot shock stress decreased body weight, impaired spatial learning, and memory declined hippocampal LRP1 mRNA expression and enhanced hippocampal RAGE mRNA expression in the rat model of AD.

Weight loss and malnutrition have been identified as possible consequences of AD in studies. Additionally, weight loss is associated with rapid memory loss in patients with AD, emphasizing the importance of addressing weight loss and malnutrition in these patients (Gillette-Guyonnet et al. 2000; Kimura et al. 2019). Previously published research established that accumulating Aβ in the brain would impair the body’s weight regulation mechanism; thus, resulting in increased weight loss years before AD diagnosis (Rabin et al. 2020). Further analyses revealed that ICV-STZ would cause syndromes mimicking the sporadic AD in the rats and thus produce an imbalance in the cerebral energy and declining body weight (Paidi et al. 2015). The present research found animal weight loss following STZ injection into ventricles, similar to the observations reported by other researchers (Paidi et al. 2015; Khalili and Hamzeh 2010). On the other hand, stress has been shown to alter body weight and food intake in animal models (Geiker et al. 2018; Moreira et al. 2016). The brain contains a complex network of nuclei that regulates food intake and energy homeostasis. The neural mechanisms recruited by the stress response are complex, involving multiple circuits, such as hypothalamic

Table 2 Body weight in Exp 2

| Group        | Before (Mean ± SEM) | After (Mean ± SEM) |
|--------------|---------------------|--------------------|
| Sham         | 223.71 ± 2.55       | 250.86 ± 2.09      |
| Stress       | 225.71 ± 2.91       | 246.71 ± 1.96      |
| STZ          | 223.43 ± 3.58       | 230.14 ± 6.84 *    |
| STZ + Stress | 224 ± 3.07          | 223.29 ± 2.37 *    |

*: P < 0.01 compared with stress group

#P < 0.01 compared with stress group and +P < 0.01 compared with STZ group

B: Comparison of changes in RAGE mRNA expression level in all groups. *P < 0.05 and **P < 0.01 compared with sham group and ##P < 0.01 compared with stress group
networks that regulate energy balance, brain stem networks that regulate visceral function, sympathetic and parasympathetic mechanisms, midbrain nuclei involved in reward and arousal, and cortico-limbic structures that mediate emotional and cognitive aspects of relationships (McEwen 2004; Patterson and Abizaid 2013). In addition, several peptides and hormones are produced centrally and peripherally in response to stress, and they are capable of acting on multiple hypothalamic centers to determine the eating behaviors developed in response to a stressor (Anisman and Matheson 2005; Bali and Jaggi 2015b; Patterson and Abizaid 2013). According to some research, foot shock stress has been shown to increase circulating plasma glucose, insulin, and leptin in rats, indicating an anorectic hormonal profile (Farias-Silva et al. 2002; Griffiths et al. 1992; Patterson and Abizaid 2013). Farias-Silva et al. (2002) reported that insulin sub-sensitivity in adipose tissue is responsible for the hyperglycemia and hyperleptinemia seen in rat subjected to foot shock (Farias-Silva et al. 2002). Consistent with our findings, multiple studies have demonstrated that chronic stress has no discernible effect on body weight compared to controls (Li et al. 2009; Liao et al. 2013). At the same time, several studies have found that chronic exposure to certain types of stressors causes either a decrease (Jeong et al. 2013; Quan et al. 2011) or an increase (Geiker et al. 2018; Scott et al. 2012) in body weight. Diverse outputs may result from stress intensity, type, or duration variations. Therefore, numerous transmitters, peptides, and hormones are produced
directly or indirectly in response to stressful stimuli and AD (Bloch et al. 2017; Patterson and Abizaid 2013). Recent research indicates that specific cognitive dysfunctions in Alzheimer's patients may result in harmful nutritional conditions (Gu et al. 2014). Furthermore, stress disrupts food intake and is thought to be a substantial physiological shift in both humans and animals in reaction to stress (Bali and Jaggi 2015b; Vallès et al. 2000). Intriguingly, our study found that rats lost a significant amount of weight following the accumulation of electric foot shock stress and STZ administration. Our study revealed that the combination of electric foot shock stress and STZ administration resulted in significant weight loss in rats, which is intriguing because stress can significantly exacerbate AD symptoms. Both Exp1 and Exp2 demonstrate this weight reduction. Due to differences in study design, the weight loss of the Exp2 group is significantly greater than that of the Exp1 group.

According to the studies in the field, learning and memory deficits have been proposed as the prime issues in AD, which prevent patients from enjoying everyday life (Jahn 2013; White and Ruske 2002). The results of our study and previous investigations confirmed a lower level of spatial learning and memory in the ICV-STZ animals on MWM (Paidi et al. 2015; Negintaji et al. 2015; Sasaki-Hamada et al. 2019). On the other hand, chronic stress would stimulate the enhanced levels of glucocorticoid stress hormones, which have detrimental impacts on the function and structure of the CNS, particularly the hippocampus (Conrad 2010; Shors 2004). We showed that chronic electric foot shock for ten days induced memory impairment in normal rats that matched earlier findings (Moosavi et al. 2007; Wright and Conrad 2008). Although there is insufficient information on the etiology of more common (sporadic) forms of AD, previous research indicated that the interaction of environmental risk factors and genetic backgrounds plays a significantly influential role in the onset and progression of sporadic AD. Epidemiological studies have identified stress as a risk factor for AD. The evidence suggested that AD would impair the normal functioning of the hypothalamic–pituitary–adrenal axis. The enhanced level of glucocorticoid can hit the hippocampus due to high levels of glucocorticoid receptors in the hippocampus of its neurons. Earlier research indicated that glucocorticoids and stress increased APP, BACE, and C99 levels, implying that stress induces APP processing along the amyloidogenic pathway, resulting in increased plaque formation and accelerating the neuropathology of AD, including increased Aβ deposition in the hippocampus (Cuadrado-Tejedor et al. 2012; Dong and Csernansky 2009; Green et al. 2006). Also, studies conducted during the last decade found a relationship between levels of amyloid deposition and memory performance (Ford et al. 2015; Ramírez et al. 2018). Hence, diverse spatial tasks have been utilized for assessing hippocampal functions, and MWM has been introduced as one of the classical tests of spatial learning and memory for rodents. A majority of the MWM investigations reported that chronic stress impaired spatial memory in the rat model of AD (Conrad 2010; Vorhees and Williams 2006). What was clear based on our findings is a considerable decline in spatial learning and memory when STZ coincided with chronic stress.

There is a correlation between neurological disorders, cerebrovascular dysfunction, and BBB function changes (Montagne et al. 2017). Moving Aβ across BBB needs a professional transport system. Notably, LRP1 and RAGE...
receptors contribute significantly to the free un-bound Aβ between blood and brain and across BBB (Deane 2012; Sagare et al. 2012). Moreover, neurodegenerative illnesses like AD showed more significant levels of RAGE and lower levels of LRP1 related to the Aβ toxicity-induced damages on the brain microvasculature (Erickson and Banks 2013). Wang et al. (2017) demonstrated an increase in RAGE and a decrease in LRP1 protein levels in the hippocampus of an STZ-induced AD rat model (Wang et al. 2017). In this sense, the present research confirmed the results obtained from earlier experiments. On the other hand, some investigations studied the impact of chronic and acute stress on the BBB functions (Kempuraj et al. 2019). A limited number of studies reported the correlation between chronic stress and BBB transport system dysfunction markers (Kempuraj et al. 2019). Our research revealed chronic electric foot-shock stress-induced lower LRP1 expression and greater RAGE expression in the rats’ hippocampus. Based on the results of the previous studies, stress can exacerbate other disorders like depression (Pfleger et al. 2015), metabolic syndrome (Tamashiro et al. 2011), and diabetes (Pavlatou et al. 2008). Previous research has indicated that RAGE increased and LRP1 decreased at the BBB of STZ-induced diabetic rats. The results revealed that the upregulation of RAGE and down-regulation of LRP1 expression at the BBB contribute to Aβ deposition in diabetes mellitus (Hong et al. 2009; Liu et al. 2009). Additionally, Franklin et al. (2018) found that chronic unpredictable stress increased the mRNA levels of RAGE and high mobility group box one protein (HMGB1) in enriched hippocampal microglia. Furthermore, they demonstrated that RAGE deletion mutant mice are resistant to chronic unpredictable stress-induced behavioral deficits (Franklin et al. 2018). On the other hand, Wang et al. (2020) evidenced that chronic unpredictable mild stress increased hippocampal LRP1 expression in a depressive-like adult male rat model (Wang et al. 2020). The current research findings confirmed that electrical foot shock stress decreases LRP1 expression. The differences could be attributed to various factors, including the stress paradigm type and timing of stressors used in the studies and the methodology used.

Several elements of the current study merit remark, which should be considered in future research. Numerous factors, for example, were not quantified, such as glucocorticoid levels in the blood or Aβ deposits in the brain. Additional research should be conducted on the effects of various types of stress protocols on AD models. Furthermore, these findings can be applied to other behavioral tasks (including the Elevated plus maze test), and large-scale studies should be considered to further establish correlations between stress and BBB dysfunction.

Conclusion

According to mounting evidence, chronic stress exposure is a risk factor for people with AD, adversely affecting the disease’s course. In previous studies, chronic stress has been shown to sensitize brain cells to subsequent challenges, and severe stress elicits synergistic toxicity in response to a second challenge. In other words, subsequent exposure of the preconditioned organism to stress stimuli can increase the brain’s susceptibility to AD. Investigating the underlying mechanisms of stress-induced brain vulnerability provides a foundation for understanding the relationship between the efficacy of stress interventions and neurodegenerative disorders such as Alzheimer-related memory decline. According to our findings, stress prior to (Exp1) and following (Exp2) ICV-STZ injection exacerbates LRP1 reduction and RAGE enhancement in the hippocampus. The study results pave a new way for scientists to develop novel strategies for AD treatment.

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