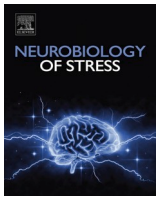
Neurobiology of Stress 33 (2024) 100685

Contents lists available at ScienceDirect

Neurobiology of Stress

journal homepage: www.elsevier.com/locate/ynstr



Dopamine and D1 receptor in hippocampal dentate gyrus involved in chronic stress-induced alteration of spatial learning and memory in rats

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ARTICLE INFO

Handling Editor: Dr. Rongjun Yu

Keywords:
Chronic stress
Hippocampal dentate gyrus
Dopamine
D1 receptor
Learning and memory
Rats

ABSTRACT

There is increasing evidence that chronic stress (CS), which occurs when the body is exposed to prolonged stressors, significantly impairs learning and memory. Dopamine (DA) plays a critical role in learning and memory in the hippocampus through the activation of D1-like receptors (D1R). However, the specific roles of DA and D1R in the hippocampal dentate gyrus (DG), particularly in CS-induced changes in spatial learning and memory, are not well understood. In this study, we established a CS rat model through the random application of various stressors. We assessed spatial learning and memory using the Morris water maze (MWM) and measured DA concentration and the amplitude of field excitatory postsynaptic potentials (fEPSP) in the DG during the MWM test in freely moving rats. We also examined the involvement of D1R in spatial learning and memory by microinjecting its antagonist (SCH23390) into the DG, and then analyzed the expressions of phosphorylated (p-) Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), protein kinase A (PKA), and cAMP-response element binding protein (CREB) in the DG using Western blot. During the MWM test, compared with the control group, the escape latency was increased, and the percentage of distance in target quadrant and the number of platform crossings were decreased, in addition, the increase of fEPSP amplitude in the DG was significantly attenuated in CS group. In the control group, the DA concentration in the DG was significantly increased during the MWM test, and this response was enhanced in the CS group. Microinjection of SCH23390 into the DG significantly improved the spatial learning and memory impairments in CS rats, and reversed the inhibitory effect of CS on increase of fEPSP amplitude in the DG during the MWM test. Furthermore, SCH23390 partially reversed the inhibitory effects of CS on the expressions of p-CaMKII, p-PKA, and p-CREB in the DG. Our findings suggest that overactivation of the DA-D1R system in the hippocampal DG impairs spatial learning and memory and related synaptic plasticity in CS rats via downregulation of PKA-CREB signaling pathway.

1. Introduction

In everyday life, people are often exposed to internal or external stressor that affects their well-being and physical health. Chronic stress (CS) is essential for maintaining normal life activities, as moderate levels can have positive and beneficial effects on the body. However, when CS becomes prolonged or intense, it can have detrimental effects, leading to functional changes in the cardiovascular system (Kivimaki and Steptoe, 2018), central nervous system (Asakura et al., 2000), digestive system (Stefanaki et al., 2023) and endocrine system (Zefferino et al., 2021), as well as affecting learning and memory (Knauft et al., 2021; Lupien et al., 2009). Furthermore, ample evidence indicates that CS can have varying effects on learning and memory, either impairing or enhancing cognitive function depending on the nature of the stressor and the individual experiencing it (Chaby et al., 2015; Woo et al., 2018).

The hippocampus is a critical structure involved in various learning

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https://doi.org/10.1016/j.ynstr.2024.100685
Received 31 May 2024; Received in revised form 7 October 2024; Accepted 23 October 2024
Available online 24 October 2024

and memory processes, including episodic memory, semantic memory, and spatial navigation (Ayhan et al., 2021; Bartsch and Wulff, 2015). In rodents, spatial learning and memory serve as essential behavioural paradigms for understanding hippocampal memory mechanisms. Histomorphologically, the hippocampus is primarily divided into the CA1 and CA3 regions, the dentate gyrus (DG), and the inferior gyrus (Lathe et al., 2020). DG serves as a key preprocessor, receiving glutamatergic inputs from the entorhinal cortex and playing a central role in encoding spatial information (Jonas and Lisman, 2014). It is well-established that glutamate (Glu) and N-methyl-D-aspartate (NMDA) receptor-mediated signaling are crucial for long-term potentiation (LTP) as well as hippocampal spatial learning and memory processes (Volianskis et al., 2015). Activation of NMDA receptors (NMDAR) lead to an increase in intracellular Ca^{2+} levels, which in turn triggers the phosphorylation of cAMP response element-binding protein (CREB) through the activation of various phosphatases and kinases, including Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) (Hell, 2023). Phosphorylated (p-) CREB promotes the expression of brain-derived neurotrophic factor (BDNF), a critical regulator of neuronal survival, growth, synaptic plasticity, and long-term memory (Huo et al., 2015; Liu et al., 2021). Furthermore, studies have shown that CS significantly increases Glu levels and upregulates the expressions of the NMDAR subunits NR2B and metabotropic glutamate receptor 1 (mGluR1) in the hippocampus (Chen et al., 2013). In addition, our previous results suggest that overactivation of Glu-NMDAR in the hippocampal DG is associated with impairments of spatial learning and memory in the obese rats (Lv et al., 2024).

Dopamine (DA) is a crucial neurotransmitter and modulator within the central nervous system (CNS). Numerous studies have demonstrated that DA in the hippocampus plays a significant role in regulating learning and memory, and synaptic plasticity (Broussard et al., 2016; McNamara et al., 2014). Two major types of DA receptors have been identified in the CNS: the D1 and D2 receptor families (Manahan-Vaughan and Kulla, 2003). The D1 receptor (D1R) is widely expressed in the DG, and activation of DA-D1R enhances hippocampal learning and memory under physiological conditions (Hamilton et al., 2010; Mu et al., 2011). However, excessive dopaminergic stimulation can impair hippocampal reference and spatial memory, an effect that can be reversed by the D1R antagonist SCH23390 (Bezu et al., 2017; Ramires Lima et al., 2021). Moreover, we have found that DA and the D1R in the DG enhance hippocampal spatial memory and LTP by regulating glutamate signaling (F. Wang et al., 2019). However, the role of DG-containing DA and D1R in the CS-induced alterations in spatial learning and memory have not been reported. Therefore, in this study, we established a CS rat model through the random application of various stressors, and measured both DA release - using an *in vivo* microdialysis method- and synaptic efficiency in the DG during Morris water maze (MWM) test in freely-moving rats. D1Rs antagonist was then used in the DG to examine its effects on spatial learning and memory deficits in CS rats.

2. Methods

2.1. Animals and chronic stress

A total of 40 male Sprague-Dawley rats (12 months old) with average weight of 380 ± 30 g was obtained from Experimental Animal Department (YanBian University). Rats were housed individually at the animal facility for 1 week prior to beginning any experiment. The animals were randomly divided into control group and CS group, and CS rats were received a kind of unpredictable stressors every day for 30 days. There are seven types of stressors were used in this study (Table 1), and the same stressor would not occur for two consecutive days. Briefly, they include no food or water, wet litter, no bedding, noise, foot shock, tail clamp and restrained in a cylindrical plastic tube. All experiments were conducted according to the NIH Guide for the Care and Use of Laboratory Animals and the ethical regulations of Yanbian University. All efforts were made to minimize animal suffering and the number of animals used.

Table 1
The stressors used in this study.

Number	Stressors
1	no food and no water for 24h
2	wet litter for 24h
3	no bedding for 24h
4	noises (80–90 dB; 2 min apart; 3min/times; 5times)
5	foot shock (10 mA; 1 min apart; 10s/times; 30times)
6	clamp tail (Steel clamp; clamp the end of the tail 1 cm; 1min)
7	restraint in a cylindrical plastic adjustable restraint tube (25 cm long, 7 cm outer diameter) for 2h

2.2. Behavioral assessments

Morris water maze task: The spatial learning and memory abilities of the rats were assessed using the MWM task, which included place navigation trial and spatial probe trial. A circular pool (diameter: 170 cm; height: 60 cm) made of plastic with the inner surface painted black (Taimeng, China) was filled with opaque water (22 ± 2 °C). The pool was divided equally into four quadrants, and a small circular platform (diameter: 10 cm) was placed in the center of the third quadrant and submerged 5 cm beneath the water surface. The escape latency, swimming speed, swimming track, and number of platform crossings were measured by the tracking system software (SMART v3.0, Panlab, spain). In the place navigation trial, hidden platform training was consecutively performed for 4 days and each daily training session included four training trials. For each trial, rats were randomly placed into the water of one quadrant facing the wall, and were allowed to find the platform within 120 s and rest on the platform for 15 s. If a rat failed to find the platform and climb on the platform within the time limit, it led to the platform where it stayed for 15 s, and its escape latency was recorded as 120 s. The spatial probe trial was performed on the 5th day of the MWM test. Circular platforms were removed from the pool, and rats were placed into the water at a given location to swim freely for 120 s, and then the proportion of total swimming distance in each quadrant and the number of platform crossings were recorded.

Open field test: Spontaneous locomotor activity and anxiety-like behaviour were measured using the open field test (OFT). animal behavior recording and analysis system was used to track the activity of rats in a given period of time. The experimental apparatus of the OFT was a black cube opaque box 45 cm × 150 cm × 20 cm in size, the range from the inner wall edge to 20 cm is the edge area, and the rest is the central area. Each rat was placed in the center with its back facing one side of the box wall and allowed to explore freely for 5 min, and the surrounding environment was kept quiet. The total distance, distance in the center and the proportion of total time spent in the center were recorded.

2.3. Measurements of DA and field excitatory postsynaptic potentials (fEPSP)

The experiments were conducted according to the detailed procedure described previously (Ren et al., 2022; F. Wang et al., 2019). Briefly, a guide cannula affixed a 20-gauge stainless steel tubing was stereotaxically implanted 1.0 mm above the DG region and a bipolar stimulating electrode was lowered into the ipsilateral perforant path (PP); and then the animals were individually housed and allowed to recover from surgery for 2 days. After recovery, a microdialysis probe or a microinjection tube was inserted through the guide cannula into the DG region, and a monopolar recording electrode inserted into the 20-gauge stainless steel tubing reached the DG, where fEPSP evoked by stimulation of the PP were recorded. On the day after insertion of the microdialysis

probe, the measurements of DA concentration and fEPSP amplitude in the DG were carried out during the behavioral test under freely-moving conditions. The microdialysis probe was perfused with modified Ringer's solution at a constant rate of 1.5 μL/min, and the perfusate from the DG was automatically injected into the HPLC detection system (HTEC-500; Eicom, Japan) to measure the DA concentration. The measurement of fEPSP amplitudes was performed simultaneously: the PP was stimulated 15 times by single-phase square wave pulses (0.1ms/phase, intensity was chosen to elicit 50% of the maximal fEPSP, interval was 30s) generated with the flexible stimulus isolator (ISO-Flex; A.M.P.I., Jerusalem, Israel). Evoked responses were filtered (0.5–2.0 kHz) and amplified (1000 ×) by an AC amplifier (Neurolog, Digitimer, UK), digitized (Micro3, CED, UK), and analyzed on a computer with Spike2 software (CED, UK).

2.4. Plasma collection and enzyme-linked immunosorbent assay (ELISA)

After the end of the spatial probe trial of the MWM test, the animals were anesthetized with isoflurane. The pleural cavity was opened at the xiphoid process to expose the heart, the right ventricular blood (about 1–2 ml) of the rats was collected through anticoagulant vacuum sampling and sacrificed by rapid decapitation. After blood collection, the sample tube were placed upright at 4 °C for 30 min, and then centrifuged at 3000 rpm for 20 min. The plasma was collected, and the ELISA was performed using an ELISA kit (Wuhan huamei Co., Ltd., Wuhan, China), according to the manufacturer's instructions. The absorbance was measured at 450 nm by an enzyme-labeling instrument. The concentrations of corticosterone (CORT) and epinephrine (E) in the plasma were calculated according to the absorbance of CORT and E based on a standard curve.

2.5. Western blot assessments

The hippocampal DG region was quickly dissected out from brain on ice-cold surface, and the Western blot analysis was carried out (Wang et al., 2020). Bradford Protein Assay kit (Beyotime Biotechnology Co., Ltd., Shanghai, China) was used to obtain total protein from the DG sample. Equal amounts of protein were separated by SDS-PAGE before electrotransferred to polyvinylidene difluoride (PVDF) membranes. The PVDF membranes were blocked with 5% (w/v) skimmed milk at room temperature (RT) for 2 h, and then incubated with anti-D1R, anti--CaMKII, anti-p-CaMKII, anti-p-PKA anti-CREB, anti-p-CREB, (1:1000; Cell Signaling Technology, USA) and anti-β-actin (1:2000; Bioss, Beijing, China) at 4 °C overnight. The membranes were washed three times in PBST and incubated with secondary anti-bodies (1:2000) at RT for 1 h. After extensive washing with PBST, bands were visualized with the ECL Plus Western blotting detection system (ComWin Biotech, Beijing, China) and then quantified using Biospectrum 615 imaging system.

2.6. Drug

SCH23390 (Sigma, St Louis, Missouri, USA), a D1R antagonist, that was dissolved in saline (1 μg/μL) and stored at 4 °C. A microinjection tube was connected to the microinjection pump (ESP-64, Eicom, Japan) on each behavioral test day, and 1.0 μL of the drug solution was injected into the hippocampal DG 30 min before the behavioral test.

2.7. Statistical analysis

Data were presented as mean ± SEM. Data were statistically analyzed using GraphPad Prism software. Statistical significance was evaluated by *t*-tests, one-way ANOVA and two-way ANOVA. Results with $P < 0.05$ were considered significant.

3. Results

3.1. CORT and E concentrations in plasma

In the CS group, the concentrations of CORT (Fig. 1A, $t_{(14)} = 8.060$, $P < 0.05$) and E (Fig. 1B, $t_{(14)} = 4.987$, $P < 0.05$) in the plasma of rats were significantly higher than those in the control group, indicating that the CS animal model was successfully prepared.

3.2. Spontaneous locomotor activity and anxiety-like behaviour

After CS model was successfully prepared, spontaneous locomotor activity and anxiety-like behaviour of rats were observed by OFT. As shown in Fig. 2B, there was no significant difference in the total moving distance between the two groups ($t_{(14)} = 0.7695$, $P > 0.05$), indicating that CS did not affect the spontaneous motor activity of rats. The results also showed that there was no significant difference between the two groups in residence time (Fig. 2C, $t_{(14)} = 1.1052$, $P > 0.05$) and moving distance (Fig. 2D, $t_{(14)} = 0.8277$, $P > 0.05$) in the central area, suggesting that CS rats had no anxiety-like behavior.

3.3. Spatial learning and memory abilities

During the 4 days of the place navigation trial of the MWM test, the escape latency decreased in both control and CS groups over the course of the training days, however, on the 1st–3rd days, escape latencies were significantly longer in CS group compared with control group (Fig. 3A, $F_{(3, 56)} = 28.68$, $P < 0.05$). In addition, there was no significant difference in the daily swimming speed between the two groups (Fig. 3B, $F_{(3, 3)} = 1.953$, $P > 0.05$).The spatial probe trial of MWM test was carried out on the 5th day, compared with control group, the proportion of swimming distance in the target quadrant (Fig. 3C, $t_{(14)} = 2.537$, $P < 0.05$) and the number of the platform crossings (Fig. 3D, $t_{(14)} = 2.256$, $P < 0.05$) in CS group were both significantly decreased.

3.4. Synaptic efficiency in the hippocampal DG

LTP is not only induced by high-frequency electrical stimulation in various subregions of the hippocampus, but also accompanied by LTP-like synaptic transmission effect during learning and memory called learning dependent LTP (LD-LTP). In the present study, LD-LTP in the DG during spatial learning and memory of rats was observed with the increase of fEPSP amplitude. In addition, "basal levels" indicate the values obtained before starting behavioral test in each parameter, and the fEPSP amplitude in the DG during the MWM test was expressed as the percentage of the basal level. In control group, the fEPSP amplitude in the DG was markedly increased on the 3rd day of the MWM test compared with basal level (Fig. 4A, $F_{(1, 60)} = 8.187$, $P < 0.05$); in contrast, in CS group, there was no significant change in the fEPSP amplitude during the MWM test. In addition, compared with control group, the fEPSP amplitude in the DG was significantly decreased in CS group on the 3rd day of MWM test (Fig. 4A, $t_{(10)} = 2.537$, $P < 0.05$).

3.5. DA concentration and D1R expression in the DG

The extracellular concentration of DA in the hippocampal DG was determined by *in vivo* brain microdialysis and HPLC techniques. As shown in Fig. 4B, compared with the basal level, the concentration of DA in the DG was significantly increased on the 3rd day of MWM test in control group (Fig. 4B, $F_{(1, 60)} = 6.955$, $P < 0.05$), in contrast, the concentrations of DA in the DG in CS group were significantly increased on the 2nd and 3rd days of the MWM test (Fig. 4B, $F_{(5, 60)} = 3.513$, $P < 0.05$). In addition, the concentrations of DA in the DG of cs group were significantly increased compared with control group on the 2nd (Fig. 4B, $t_{(10)} = 2.261$, $P < 0.05$) and 3rd day (Fig. 4B, $t_{(10)} = 2.217$, $P < 0.05$) of MWM test. Furthermore, compared with control group, the expression of

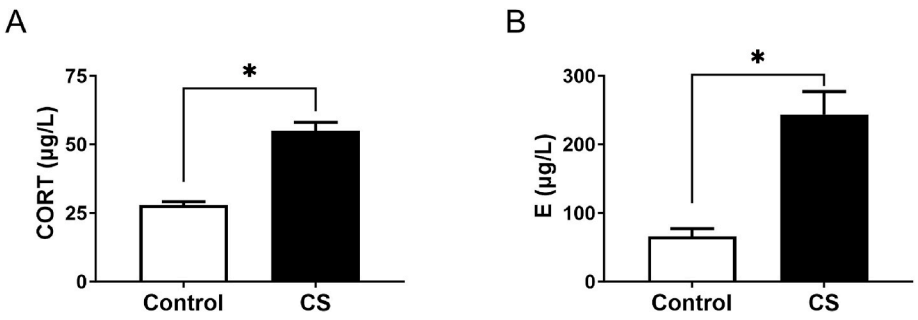

Fig. 1. Concentrations of CORT and E in the plasma. A) CORT concentration was increased in the CS rats compared to control group (*$P < 0.05$). B) E concentration was increased in the CS rats compared to control group (*$P < 0.05$). Data are expressed as mean ± SEM, n = 8.

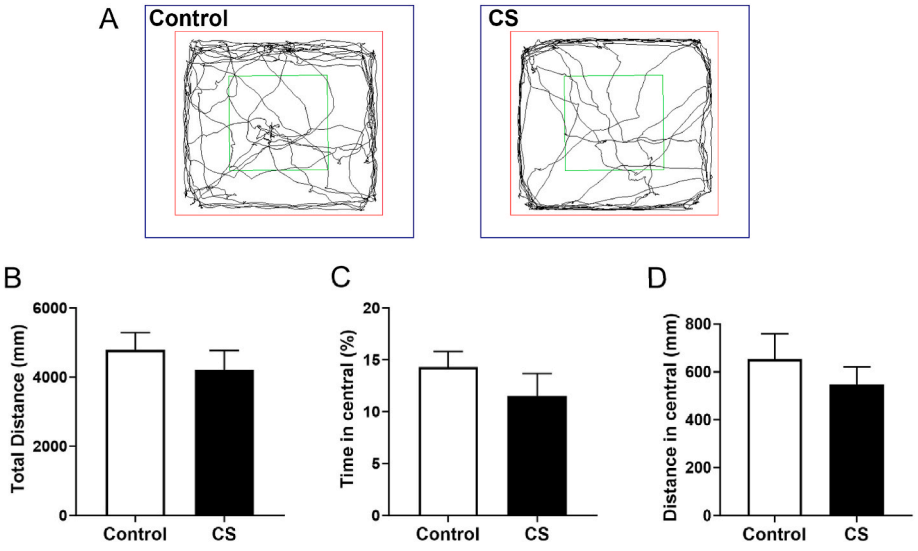

Fig. 2. Spontaneous locomotor activity and anxiety-like behaviour of rats. A) Representative traveling traces from OFT taken by each group rats. B) Total traveling distance are not significantly different between two groups. C) The proportion of time stayed in center area are not significantly different between two groups. D) Traveling distance in center are not significantly different between two groups. Data are expressed as mean ± SEM, n = 8.

D1R in the DG of CS group was significantly increased (Fig. 4C, $t_{(6)} = 4.461$, $P < 0.05$).

3.6. Effect of SCH23390 on spatial learning and memory in CS rats

To investigate the possible involvement of D1R in the DG in spatial learning and memory impairment, we observed the effect of local microinjection of SCH23390 into the DG on spatial learning and memory in CS rats. During the 4 days of the place navigation trial of the MWM test, the escape latency gradually decreased by repeated training in each treatment group, and there was no significant difference in the daily swimming speed between the groups (Fig. 5A and B); however, in CS + SCH23390 group, the escape latencies on the 1st-3rd days of MWM test were significantly reduced compared with CS + vehicle group (Fig. 5A, $F_{(2,\ 21)} = 11.59$, $P < 0.05$). During the spatial probe trial of MWM test, compared with CS + vehicle group, the percentage of swimming distance in target quadrant (Fig. 5C, $t_{(14)} = 2.740$, $P < 0.05$) and the number of platform crossings (Fig. 5D, $t_{(14)} = 2.487$, $P < 0.05$) were markedly increased in CS + SCH23390 group.

3.7. Effect of SCH23390 on fEPSP amplitude and p-CaMKII expression in the DG

As shown in Fig. 4A, the LD-LTP mainly appeared on the 3rd day of the MWM test, therefore, in here, the fEPSP amplitude in the hippocampal DG was detected before training (0d) and on the 3rd day of MWM test. The fEPSP amplitudes in the DG were significantly increased on the 3rd day of the MWM test in both CON + vehicle and CS + SCH23390 groups compared with basal level. In addition, compared with CS + vehicle group, the fEPSP amplitude in the DG was significantly increased in CS + SCH23390 group on the 3rd day of the MWM test (Fig. 6A, $t_{(10)} = 4.172$, $P < 0.05$).

As shown in Fig. 6B, compared with CON + vehicle, the expression of p-CaMKII in the DG significantly decreased in CS + vehicle group ($t_{(4)} = 7.645$, $P < 0.05$), while CS + SCH23390 group was significantly increased compared with CS + vehicle group ($t_{(4)} = 5.955$, $P < 0.05$). However, there was no significant difference in the total expression of CaMKII in the DG between the three groups ($F_{(2,\ 6)} = 0.5873$, $P > 0.05$).

3.8. Effect of SCH23390 on expression of p-PKA and p-CREB in the DG

The expression of p-PKA and p-CREB in the DG of the rats was detected by Western blot (Fig. 7). The expressions of p-PKA ($t_{(4)} = 2.289$, $P < 0.05$) and p-CREB ($t_{(4)} = 14.75$, $P < 0.05$) were significantly decreased in CS + vehicle group compared with CON + vehicle group, however, compared with CS + vehicle group, the expressions of p-PKA ($t_{(4)} = 5.503$, $P < 0.05$) and p-CREB ($t_{(4)} = 6.686$, $P < 0.05$) in CS + SCH23390 group were significantly increased. However, there was no significant difference in the total expression of CREB ($F_{(2,\ 6)} = 0.1143$, $P > 0.05$) in the DG between the three groups.

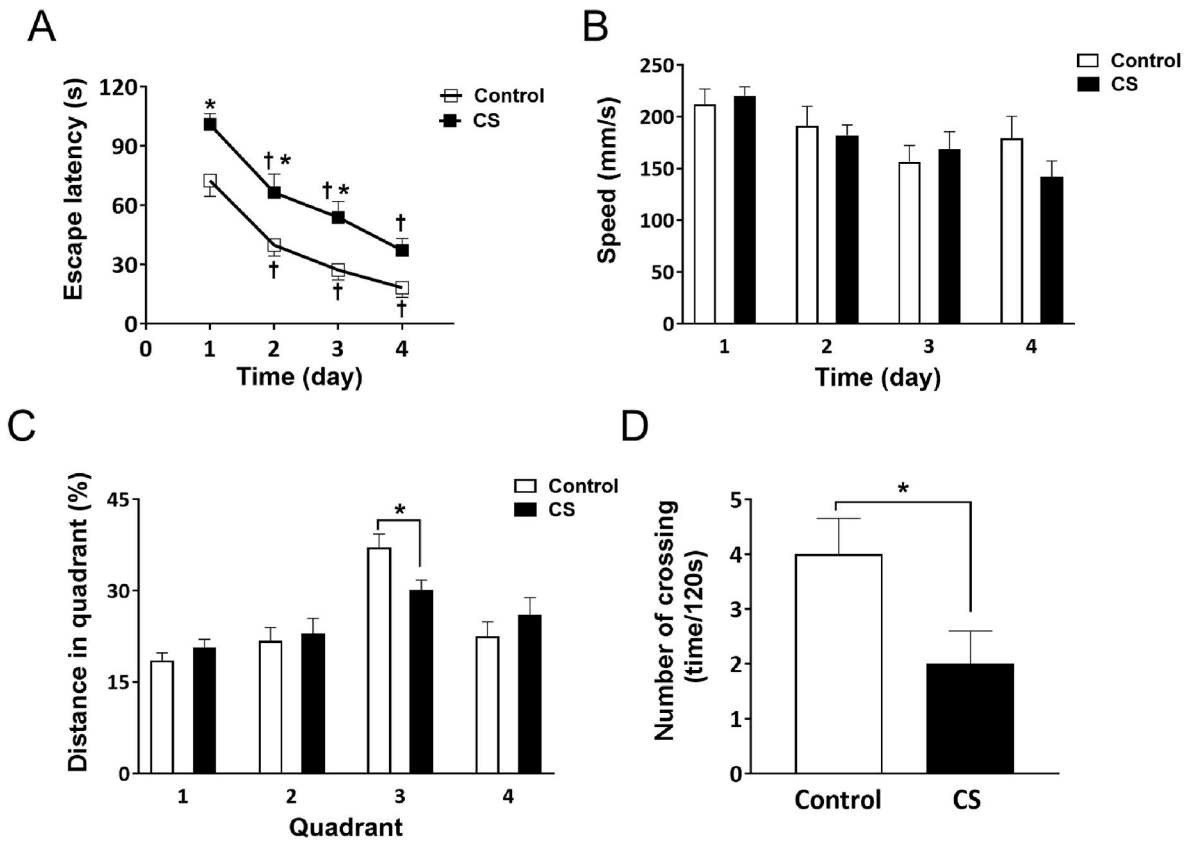

Fig. 3. Spatial learning and memory abilities. A) During the place navigation trial of MWM test, the escape latencies decreased in both control and CS groups compared to 1st day ($^{\dagger}P < 0.05$), but it showed significant increase in CS group compared to control group ($^{*}P < 0.05$). B) The swimming speed during the place navigation trial of MWM test are not significantly different between two groups. C) During the spatial probe trial of MWM test, the proportion of swimming distance in target quadrant was significantly decreased in CS group compared to control group ($^{*}P < 0.05$). D) The number of platform crossings in the spatial probe trial of MWM test was significantly decreased in CS group compared to control group ($^{*}P < 0.05$). Data are expressed as mean ± SEM, n = 8.

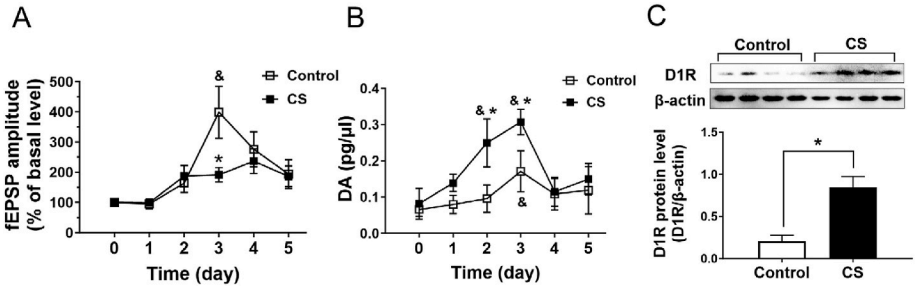

Fig. 4. fEPSP amplitude, DA concentration and D1R expression in the DG. A) fEPSP amplitude in the DG during MWM test expressed as percentages of basal level. Compared to basal level, fEPSP amplitude was increased in control group on the 3rd day of MWM test ($^{\&}P < 0.05$). Compared to control group, fEPSP amplitude was decreased on the 3rd day of MWM test in CS group ($^{*}P < 0.05$) (n = 6). B) Compared to basal level, DA concentrations in the DG were increased in control group on the 3rd day and in CS group on the 2nd and 3rd days of MWM test ($^{\&}P < 0.05$). Compared to control group, DA concentration in the DG were increased on the 2nd and 3rd days of MWM test in CS group ($^{*}P < 0.05$) (n = 6). C) D1R expression in the DG was increased in CS group compared to control group ($^{*}P < 0.05$) (n = 4). Data are expressed as mean ± SEM.

4. Discussion

4.1. Spatial learning and memory impairments in CS rats

With the rapid development of society, people are under pressure from many aspects, and various stress factors have a serious adverse impact on human health, especially middle-aged people. Therefore, this experiment focused on the possibility that middle-aged people are exposed to various unforeseen stressors all the time in life and cause CS, therefore, select 12-month-old rats (equivalent to 35–45 years old in humans) as subjects. The CS model was developed by randomly administering seven different stimuli. Under CS conditions, the sympatho-adrenomedullary system increases the release of E and activates the hypothalamic-pituitary-adrenocortical (HPA) axis, leading to an increased release of corticosteroids (CORT is main component in the rodent) to help the body adapt to stress (Wei et al., 2017). Consequently, peripheral levels of CORT and E become significantly elevated (J. Wang et al., 2019). Our data indicate that the levels of CORT and E in the plasma of CS rats were significantly higher compared to the control group, confirming successful stress induction.

CS is ubiquitous in life, and unavoidable CS has become recognized as one of the main factors affecting learning and memory functions. A

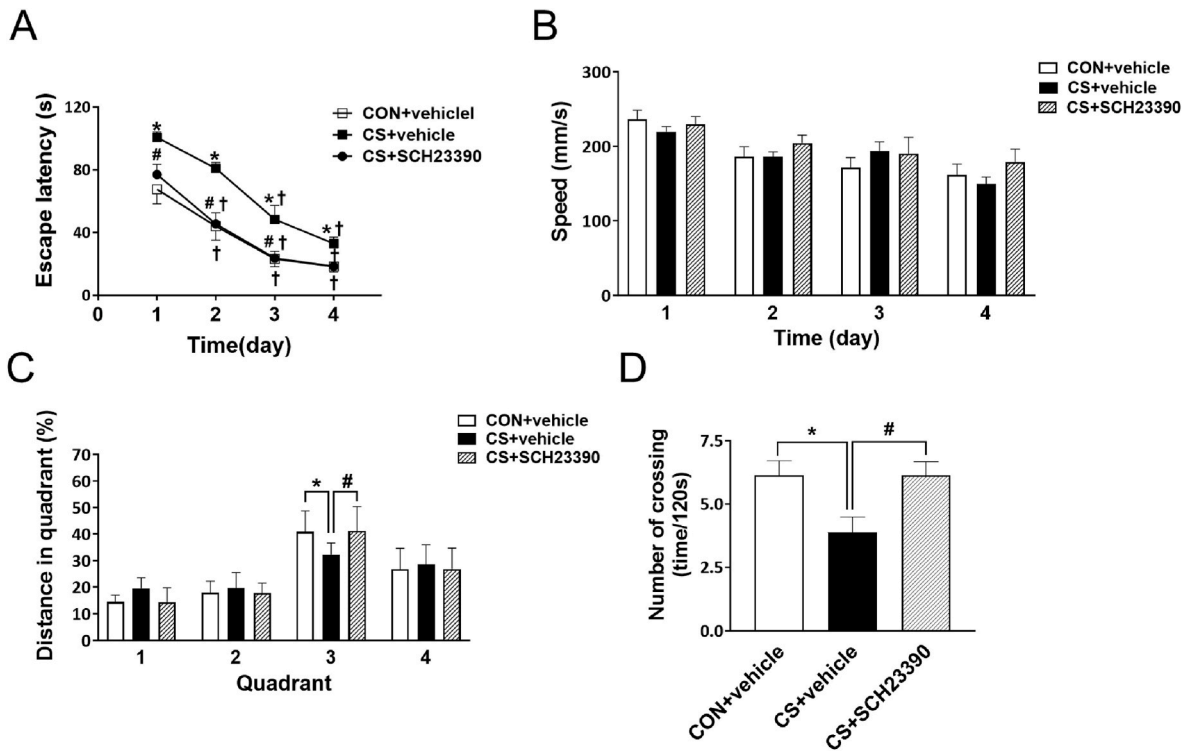

Fig. 5. Effect of SCH23390 on spatial learning and memory in CS rats. A) During the place navigation trial of the MWM test, the escape latency decreased in each group compared to 1st day ($^{\dagger}P < 0.05$). The escape latency showed significant decrease in CON + vehicle ($^{*}P < 0.05$) and CS + SCH23390 ($^{\#}P < 0.05$) groups compared to CS + vehicle group. B) The swimming speed during place navigation trial of MWM test are not significantly different between three groups. C) During spatial probe trial of the MWM test, the proportion of swimming distance in target quadrant significantly increased in CON + vehicle ($^{*}P < 0.05$) and CS + SCH23390 ($^{\#}P < 0.05$) groups compared to CS + vehicle group. D) The number of platform crossings in the spatial probe trial significantly increased in CON + vehicle ($^{*}P < 0.05$) and CS + SCH23390 ($^{\#}P < 0.05$) groups compared to CS + vehicle group. Data are expressed as mean ± SEM, n = 8.

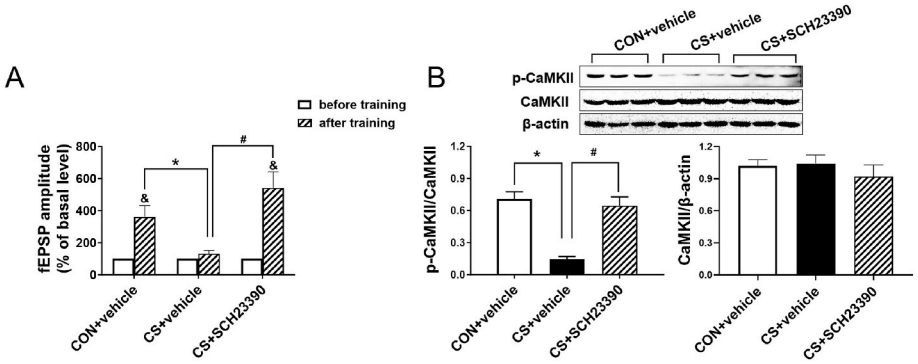

Fig. 6. Effect of SCH23390 on fEPSP amplitude and p-CaMKII expression in the DG. A) fEPSP amplitude in the DG on the 3rd day of MWM test was expressed as percentages of basal level. fEPSP amplitude increased in CON + vehicle and CS + SCH23390 groups after training compared to before training ($^{\&}P < 0.05$). fEPSP amplitude increased after training in CON + vehicle ($^{*}P < 0.05$) and CS + SCH23390 ($^{\#}P < 0.05$) groups compared to CS + vehicle group (n = 6). B) Compared to CS + vehicle group, p-CaMKII expresstions in the DG were increased in CON + vehicle ($^{*}P < 0.05$) and CS + SCH23390 ($^{\#}P < 0.05$) groups. CaMKII expression showed no significant differences (n = 3). Data are expressed as mean ± SEM.

large number of research results have shown that the effect of CS on learning and memory function is complex. For example, 6 weeks of mild unpredictable stress or 21d restraint stress can impair learning (Luo et al., 2017; Zhang et al., 2020), however, chronic restraint stress (3 h/day, 6 weeks) can promote learning and memory (Wang et al., 2020). Our results show that compared with control group, the escape latency was significantly increased, and the proportion of swimming distance and the number of the platform crossing in the target quadrant were both significantly decreased in CS group, but there was no significant difference in swimming speed between the two groups. Psychological studies have demonstrated that CS can induce emotional responses in animals, such as anxiety and depression (Wislowska-Stanek et al., 2016), which can impact learning and memory abilities (Schwabe et al., 2012). In the present study, the OFT was employed to assess spontaneous motor activity, anxiety and depression levels in CS rats, and the results showed no significant differences between the CS and control groups. These results indicated that the observed impairments in spatial learning and memory caused by CS were not related to emotional responses.

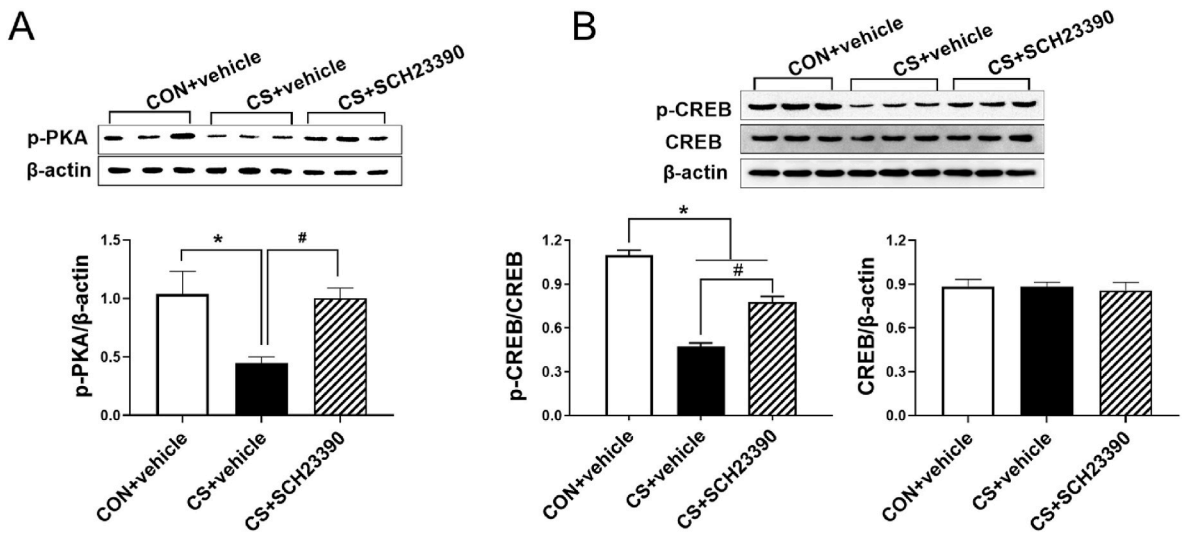

Fig. 7. Effect of SCH23390 on expressions of p-PKA and p-CREB in the DG. A) p-PKA expressions were increased in CON + vehicle ($^{*}P < 0.05$) and CS + SCH23390 ($^{\#}P < 0.05$) groups, compared to CS + vehicle group. B) p-CREB expressions in CON + vehicle ($^{*}P < 0.05$) and CS + SCH23390 ($^{\#}P < 0.05$) groups were increased compared to CS + vehicle group. CREB expression showed no significant difference. Data are expressed as mean ± SEM, n = 3.

4.2. DA and LTP in the DG in relation to spatial learning and memory impairments in CS rats

Research has shown that CS affects hippocampal learning and memory by altering neuron morphology, inhibiting neurogenesis, and reducing hippocampal volume (Price and Duman, 2020). CS also changes the firing properties of place cells in the rodent hippocampus, impacting spatial learning and memory (Kim et al., 2007). During stress, the locus coeruleus-sympathetic-adrenal medulla (LSAM) system is activated, and recent study has shown that dopaminergic axons and subsequent DA release in the dorsal hippocampus originate from neurons of locus coeruleus (LC) (Takeuchi et al., 2016). The hippocampal DG, which receives neural projections from the locus coeruleus, has a relatively dense expression of D1 receptors (Yamasaki and Takeuchi, 2017). Studies indicate that DA in the hippocampal DG promotes learning and memory and contributes to the formation and maintenance of LTP by activating D1 receptors (Daba Feyissa et al., 2019). However, the role of DA-D1R in the DG in CS-induced hippocampal memory and LD-LTP has not been fully understood. In this study, the amplitude of fEPSP in the DG was used to assess synaptic plasticity such as LTP. Previous research has shown that hippocampal LTP arises during the formation of a conditioned reflex and diminishes during extinction training (Ren et al., 2022). Importantly, both the peak of LTP and its subsequent decline precede the conditioned behavior, suggesting that LTP is learning-dependent (Kenney and Manahan-Vaughan, 2013). In the present study, the fEPSP amplitude in the DG was increased during the spatial learning process in control group, and this increase in fEPSP amplitude was significantly attenuated in CS group, furthermore, the in vivo induction of long-term enhancement on synaptic efficiency roughly related to the rats' ability to perform the MWM task. Therefore, the LTP observed in this study can be considered to be learning-related. Moreover, the results of this experiment showed that, in the control group, the DA concentration in the DG was markedly increased during the spatial learning process, and this change was accompanied by corresponding change in the fEPSP amplitude in the same region, confirming that DA in the DG may be involved in LD-LTP during spatial learning. However, in the CS group, DA response in the DG during spatial learning was significantly enhanced, while the LD-LTP in the DG was inhibited. These results suggested that DA in the DG is involved in impairments of spatial learning and memory in CS rats, may via disturbing synaptic efficiency during spatial learning.

4.3. Antagonism of D1R in the DG improves spatial learning and memory impairments in CS rats

In order to examine whether DA in the DG is involved in CS-induced impairments of spatial learning and memory via activation of D1R, SCH23390 (an antagonist of D1R) was microinjected into the DG before daily MWM training in CS rats. Our results showed that in CS rats, SCH23390 in the DG significantly improved the spatial learning and memory impairments, and reversed the inhibitory effect of CS on increase of fEPSP amplitude in the DG during the MWM test. It has been shown that the formation of LTP in the hippocampal DG depends on the activation of NMDAR and subsequent phosphorylation of CaMKII (Fedder and Sabo, 2015). Our results showed that CS significantly inhibited the expression of p-CaMKII in the DG, however SCH23390 could reverse this inhibitory effect of CS. These results suggest that D1R in the DG could mediate DA-induced spatial learning and memory impairments by inhibiting synaptic efficiency during spatial learning.

The D1R is a G protein-coupled receptor, after being activated by ligands, it activates adenylate cyclase (AC) through Gs protein, causing activation of the cAMP/PKA pathway, which in turn causes a variety of downstream target proteins (such as CREB) phosphorylation, which regulates cellular function (Luo et al., 2017). Our results showed that the microinjection of SCH23390 into the DG partly reversed the inhibitory effects of CS in the expressions of p-PKA and p-CREB in the DG. These results indicate that over-activaed DA-D1R signaling and suppression of PKA-CREB pathway in the hippocampal DG are involved in spatial learning and memory impairments in CS rats.

In conclusion, the findings of the present study suggest that DA-D1R system in the hippocampal DG is involved in the impairments of spatial learning and memory and related synaptic plasticity in CS rats, in part by inhibition of D1R downstream protein expression, including PKA and CREB.

CRediT authorship contribution statement

Linping Wang: Writing – original draft, Investigation. **Weiyao Wang:** Formal analysis, Data curation. **Yingshun Li:** Project administration. **Hua Jin:** Supervision, Funding acquisition. **Bin Xiao:** Writing – review & editing, Visualization, Validation. **Qinghua Jin:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Funding and disclosure

This work was supported by the National Natural Science Foundation of China (32160195) and Jilin Scientific and Technological Development Program (YDZJ202201ZYTS255).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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