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Original Research

Changes in depressive-like behaviors induced by spinal cord injury based on the hypothalamic-pituitary-adrenal axis and hippocampal neurogenesis

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A reduction in sucrose preference is a key characteristic of depressive-like behaviors after spinal cord injury as judged by the sucrose preference test, the hypothalamic-pituitary-adrenal axis and adult hippocampal neurogenesis. Male rats were divided into three groups: control, sham and spinal cord injury groups. The spinal cord injury rats received a severe mid-thoracic contusion. The Basso, Beattie and Bresnahan score was used to assess motor function. The sucrose preference test and forced swim test were used to evaluate depressive-like behaviors. Serum corticosterone levels were examined by enzyme-linked immunosorbent assay and hippocampal glucocorticoid receptor levels were examined by Western blot to evaluate the function of the hypothalamic-pituitary-adrenal axis. Adult hippocampal neurogenesis was assessed by testing hippocampal brain-derived neurotrophic factor and tropomyosin receptor kinase B levels by Western blot and doublecortin levels by immunohistochemistry. Data showed that spinal cord injury impaired motor function. The spinal cord injury rats exhibited decreased sucrose preference on day six, which continued to decrease until day twelve, followed by a plateau phase. Additionally, the immobility time of the spinal cord injury rats was increased on day thirty-four. Moreover, serum corticosterone levels in the spinal cord injury group peaked on day seven, was decreased by day twenty-one and was increased again on day thirty-five. Serum corticosterone levels were significantly negatively correlated with sucrose preference and positively correlated with immobility time. Finally, hippocampal doublecortin levels on days twenty-one and thirty-five were lower in the spinal cord injury group than in the other groups. These results suggest that hyperactivation of the hypothalamic-pituitary-adrenal axis and the inhibition of adult hippocampal neurogenesis may be part of the underlying mechanism responsible for depressive-like behaviors after spinal cord injury.

Keywords

Spinal cord injury; Depressive-like behaviors; Hypothalamic-pituitary-adrenal axis; Adult hippocampal neurogenesis; Sucrose test

1. Introduction

Spinal cord injury (SCI) causes severe central nervous system lesions that can affect motor and sensory functions below the site of injury. Additionally, SCI significantly impacts mental health. According to epidemiological data, the incidence of depression after SCI is as high as 63.9%, at least four to five times the rate of depression in the general population [1]. A series of studies conducted in humans and animals found that depression or depressive-like behaviors after SCI not only hinder the rehabilitation of physical function [2, 3] but also cause secondary complications such as pressure ulcers and urinary tract infection [4], increase disability and mortality and greatly reduce quality of life. Given the high incidence of depression among SCI patients and its serious adverse effects on rehabilitation and quality of life, the prevention and treatment of depression should be an important part of SCI rehabilitation. Therefore, we propose to investigate the changes in depressive-like behaviors and molecular changes after SCI to identify an ideal therapeutic target for SCI.

The causes of major depressive disorder (MDD) or other mood disorders are unknown. Evidence suggests that stressful life events are one of the main risk factors for MDD. Acute stress initially raises glucocorticoid (GC) levels and when stress becomes chronic or uncontrolled, it can result in alterations to the normal function of the hypothalamic-pituitary-adrenal (HPA) axis. This may lead to elevated glucocorticoid (cortisol in humans, corticosterone in rodents) levels and decreased glucocorticoid receptor (GR) levels (as a result of glucocorticoid resistance), which has been observed in postmortem studies and preclinical animal models [5–8]. Recent studies have detected changes in corticosterone and GR levels after SCI. Gezici *et al.* [9] and Lucin *et al.* [10] revealed that the HPA axis was activated by SCI and that corticosterone levels began to increase within 24 hours and were still elevated 35 days after SCI. Meanwhile, depressive-like behaviors were observed in SCI rats [11]. These studies have provided insight into the changes in corticosterone levels and depressive-like behaviors that occur at specific time points after SCI, but dynamic monitoring of the effect of corticosterone on depressive-like behaviors in SCI rats has not been reported. Additionally, Yan *et al.* [12] showed that

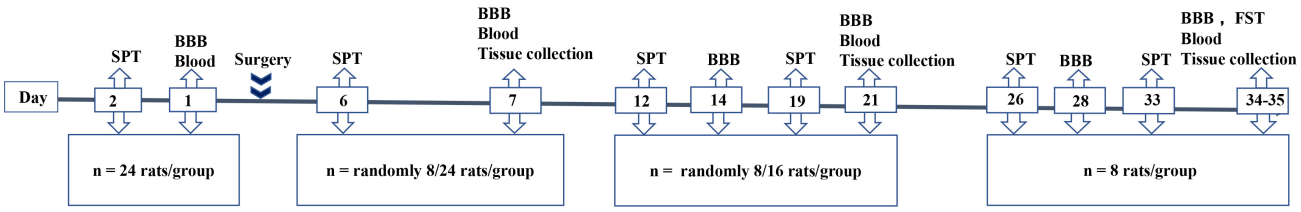

Fig. 1. Flowchart and time points in the experiment. Rats were divided into three groups: control, sham and SCI. SPT, sucrose preference test; BBB score, Basso, Beattie and Bresnahan score; FST, forced swim test. Tissue (n = 8) was collected, prepared and stored for Western blot analysis (n = 4) and immunohistochemistry (n = 4).

spinal cord GR expression level was slightly increased at fifteen minutes, peaked at eight hours, sharply declined at day one and returned to baseline on days three and seven after SCI. Nevertheless, how GR levels change after the seventh day following SCI and whether this change affects the occurrence and development of depressive-like behaviors remains unclear. Therefore, here the associations between changes in the levels of corticosterone and GR and depressive-like behaviors after SCI were explored.

High corticosterone levels have been shown to inhibit adult hippocampal neurogenesis (AHN) in the dentate gyrus (DG), which manifests as decreased cell proliferation and doublecortin (DCX) density, thus promoting depressive-like behaviors in rodents [7, 13]. Brain-derived neurotrophic factor (BDNF) and the high-affinity receptor tropomyosin receptor kinase B (TrkB) are mainly distributed in the hippocampus and involved in neuron generation, differentiation and survival [14]. The downregulation of AHN resulting from decreased hippocampal BDNF and TrkB was found to be associated with elevated glucocorticoid levels in depression [15–17]. Based on such evidence, hippocampal DCX, BDNF and TrkB can be used to monitor evidence of changes in depressive-like behaviors in SCI rats. Li *et al.* [11] observed depressive-like behaviors and the decreased expression of hippocampal BDNF on day 35 after SCI. Wu *et al.* [18] has additionally suggested that a reduction in the number of DCX-positive cells is associated with depressive-like behaviors at 16 weeks after SCI. These findings suggest that an insight into the mechanism of depressive-like behaviors after SCI can be obtained by monitoring BDNF and DCX levels. However, there have been no reports on how changes in hippocampal BDNF/TrkB and DCX at different time points after SCI affect depressive-like behaviors.

2. Materials and methods

2.1 Animals

Seventy-two male SprageDawley rats were obtained from the Experimental Animal Center of Dalian Medical University (Dalian, China). The rats were approximately 90–110 days old (weight: 260–280 g). All rats were housed individually in cages (37.5 cm [length] × 27.3 cm [width] × 16.5 cm [height]) under standard temperature (22–24 °C), humidity (40–50%), a 12-h light/dark cycle and given food and tap water *ad libitum*. All rats were allowed to adapt to the new environment for one week. Rats were randomly allocated into three groups: the (a) control (n = 24), (b) sham (received only laminectomy without SCI, n = 24); and (c) SCI (animals that received laminectomy and SCI, n = 24). All animal procedures and care were conducted according to the institutional guidelines of the local Ethics Committee for Animal Research at Dalian University and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The Ethics Committee of the Affiliated Zhongshan Hospital of Dalian University approved the study (reference number, 2017082). A flow chart of the study process is shown in Fig. 1.

2.2 Surgery and spinal cord contusion injury

Following anesthesia, a laminectomy was performed at T10 to expose a circle of the dura. Twenty-four rats received severe mid-thoracic contusion of the spinal cord and 24 rats were allocated to the sham group and received only laminectomy without SCI.Procedural details are given by Zhao *et al.* [19]. Twenty-four rats were assigned to a control group. Penicillin (160 mg/kg) was administered intraperitoneally for three consecutive days following surgery to prevent infection. The bladders of the rats were manually emptied twice daily until automatic micturition returned.

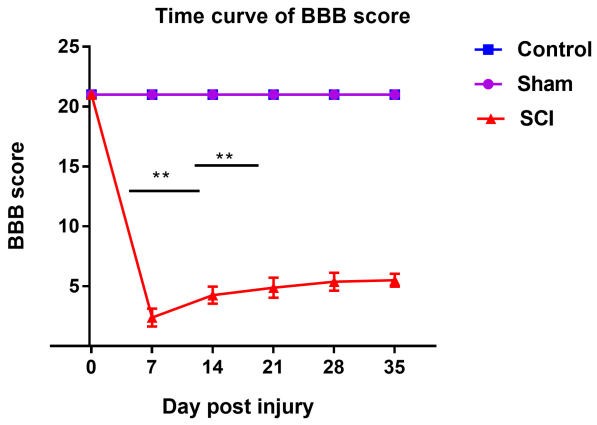

Fig. 2. Temporal profile of BBB score for each experimental group from 7 to 35 days post injury. Data are expressed as the mean ± SD (n = 8) (**$p < 0.01$).

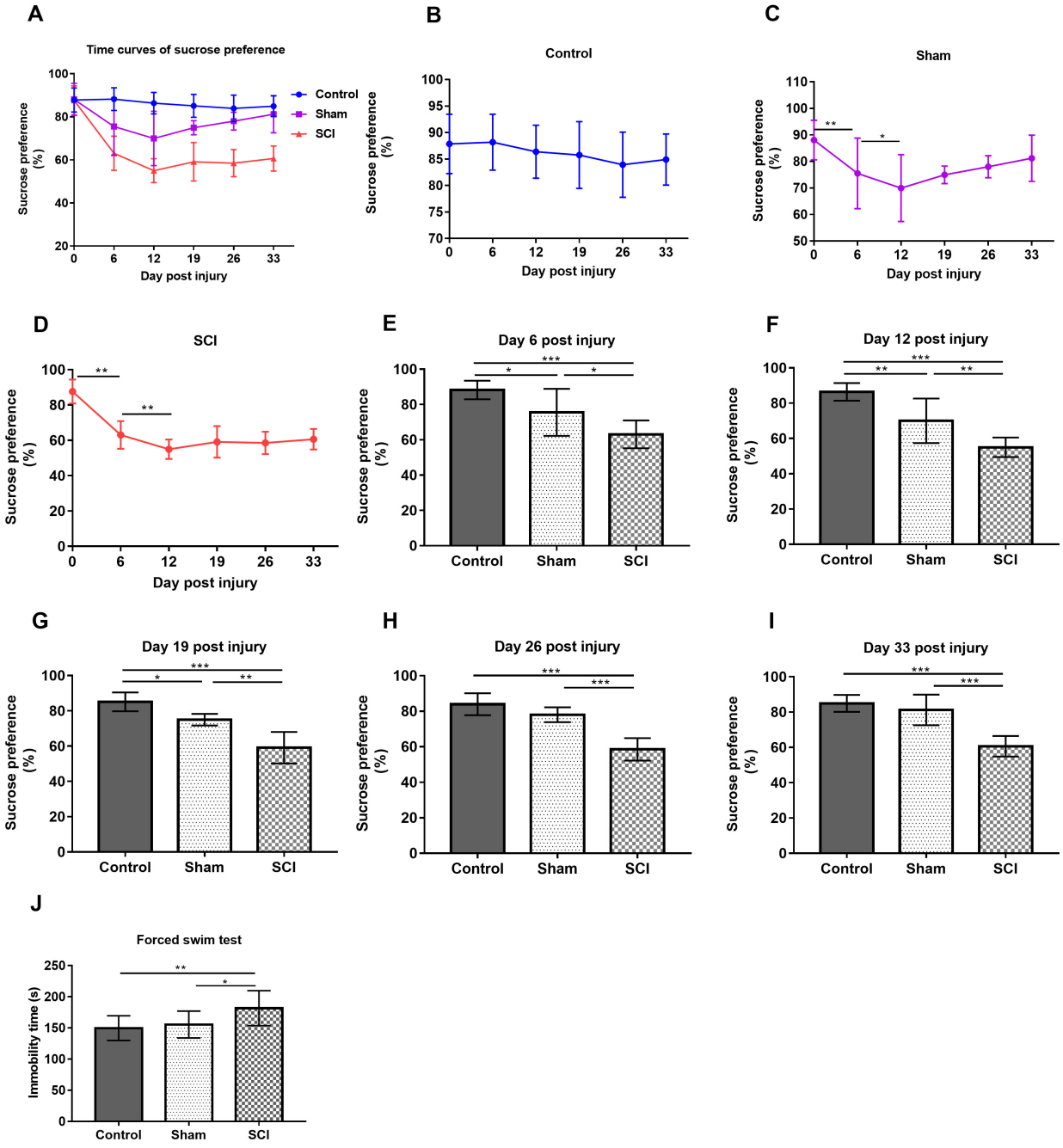

Fig. 3. Temporal profile of sucrose preference and comparative analysis of sucrose preference and forced swim test between groups. Data are expressed as the mean ± SD (n = 8). (A) Temporal profile of sucrose preference in all experimental groups. (B–D) Changes in sucrose preference in each experimental group from day 6 to day 33 post injury. (E–I) Results of comparative analysis of sucrose preference between groups from day 6 to day 33 post injury. (J) Results of comparative analysis of data from the forced swim test on day 34 post injury (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

2.3 Basso, Beattie and Bresnahan (BBB) score

Hind limb motor function was assessed in all rats by calculating the BBB score [20]. This score ranges from 0 (flaccid paralysis) to 21 points (normal gait), with higher scores indicating greater motor function. Rats were placed in an open field chamber (100 cm × 100 cm × 40 cm) without cover for four minutes. The BBB scores were determined by two observers blinded to the experimental design. The scores for both hind limbs of each rat were used to obtain an average value, which represented the motor function of that rat. The process and time points at which the BBB was determined (n = 8 rats/group) are shown in Fig. 1.

2.4 Sucrose preference test (SPT)

Two pre-weighed bottles filled with 150 mL of water and 150 mL of a sucrose solution (2%) were placed on either side of a rat's cage for two hours. To avoid position bias, as in previous studies [2], the water and sucrose bottles were switched

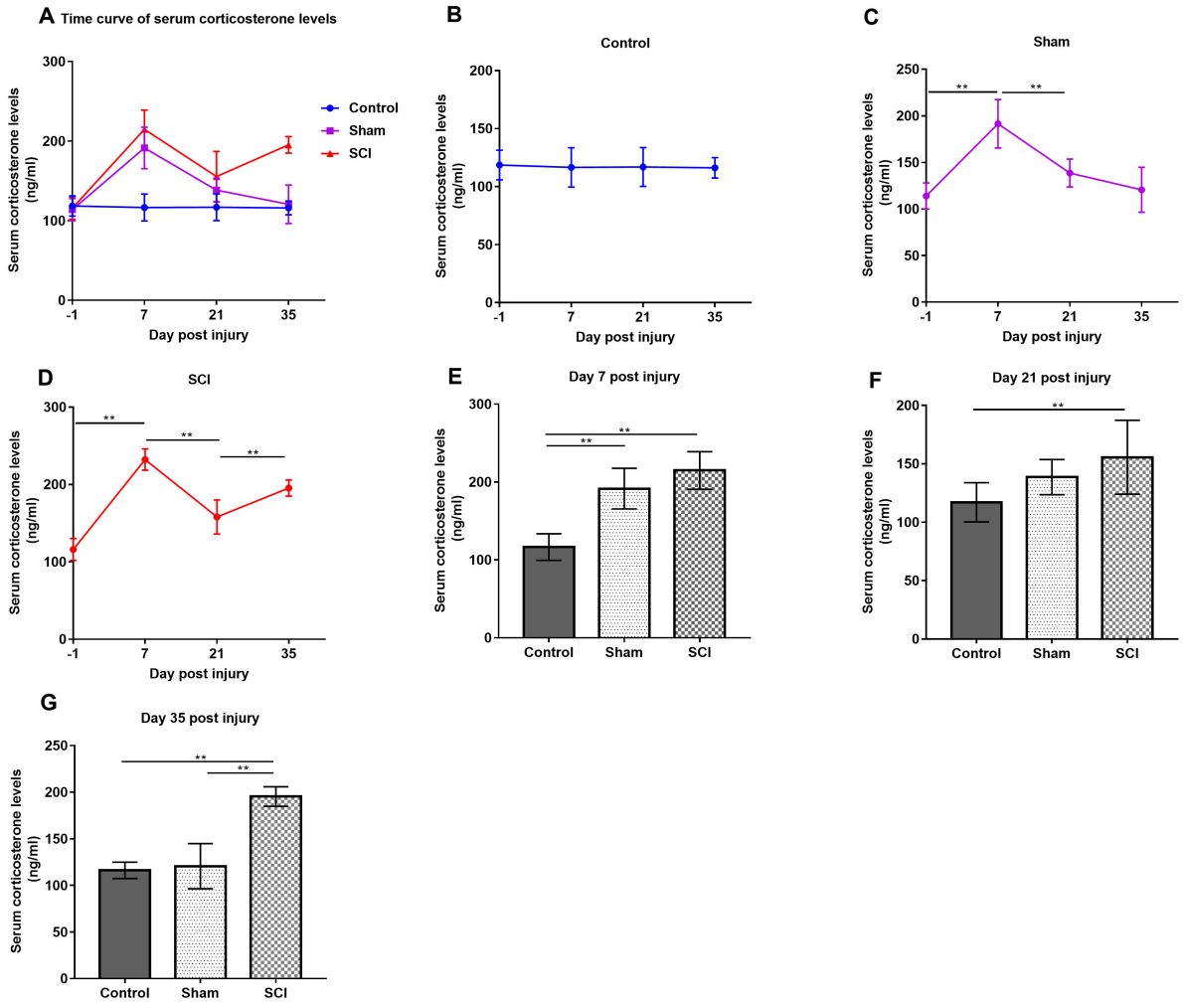

Fig. 4. Temporal profile of serum corticosterone levels and comparative analysis of serum corticosterone levels between groups. Data are expressed as the mean ± SD ($n = 8$). (A) Time curve of serum corticosterone levels in all experimental groups. (B–D) Changes in serum corticosterone levels in each experimental group from day 7 to day 35 post injury. (E–G) Results of comparative analysis of serum corticosterone levels between groups from day 7 to day 35 post injury (**$p < 0.01$).

one hour later. At the end of the test, sucrose preference was evaluated using the following formula: sucrose preference = weight of sucrose solution ingested (g)/(weight of sucrose solution ingested (g) + weight of water ingested (g)) × 100%. A reduction in sucrose preference is a key characteristic of depressive-like behavior. The process and time points at which the SPT was conducted ($n = 8$ rats/group) are shown in Fig. 1.

2.5 Forced swim test (FST)

The FST was performed on day 34 post injury, a time point at which all SCI rats had regained sufficient motor function to perform this task. Without previous adaptation to water, twenty-four rats ($n = 8$ rats/group) were placed in a cylinder (15 cm (diameter) × 40 cm (height)) filled with water (23 ± 2 °C) from which they were unable to escape, as previously described [2]. The rats were video-recorded and the cumulative immobility time during the ten minute test was analyzed. Immobility was characterized as the absence of any movement except that required to keep the head above water. Increased immobility time was assumed to indicate increased depressive-like behavior.

2.6 Enzyme-linked immunosorbent assay (ELISA)

Peripheral blood samples ($n = 8$ rats/group) were immediately collected from rats after sacrifice by decapitation on day 7, 21 and 35 post injury and centrifuged at 12,000 rpm at 4 °C for 15 min. The supernatants were collected and stored at −80 °C for subsequent determination of the serum corticosterone levels using ELISA kits (Nanjing Jiancheng Bioengineering Institute, Inc., Nanjing, China) according to the manufacturer's recommendations.

2.7 Western blot

Four rats in each group were sacrificed and hippocampal tissues were removed on days 7, 21 and 35 post injury. Western blot was employed to analyze hippocampal protein expression using standard methods. Total proteins were extracted from the hippocampus using RIPA

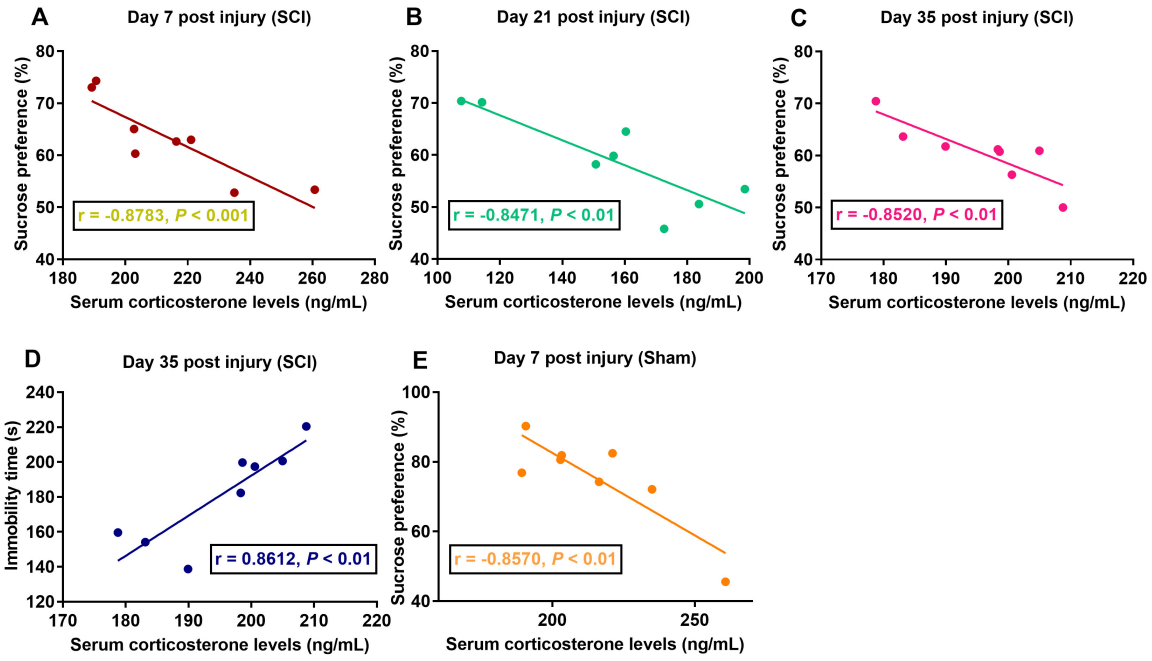

Fig. 5. Correlation analysis of serum corticosterone levels and sucrose preference. (A–C) Correlation analysis of serum corticosterone levels and sucrose preference in the SCI group on days 7, 21, and 35 post injury. (D) Correlation analysis of serum corticosterone levels and immobility time in the SCI group on day 35 post injury. (E) Correlation analysis of serum corticosterone levels and sucrose preference in the sham group on days 7 post injury. $p < 0.05$ indicates a correlation between variables. Negative and positive Pearson r values indicate that the variables were negatively or positively correlated, respectively and the closer the Pearson r was to either 1 or –1, the stronger the correlation.

buffer (Beyotime, Shanghai, China) and quantified using a BCA kit (Beyotime, Shanghai, China). Homogenized hippocampal lysates were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride (PVDF) membranes (0.45 μm, Millipore, Bedford, MA, USA). The membranes were blocked in 5% milk-TBST, washed with TBST and incubated with primary antibodies against GR (1:1000), BDNF (1:1000), TrkB (1:5000), and β-actin (1:1000) overnight at 4 °C, after which they were incubated with the corresponding secondary antibody (1:1000) for one hour. All antibodies were obtained from Abcam (Cambridge, UK). Bands were detected using enhanced chemiluminescence reagents (Beyotime, Shanghai, China) to determine protein expression, which was quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Target protein expression was calculated as the ratio of target protein gray value to the β-actin gray value × 100.

2.8 Immunohistochemistry

Four rats in each group were anesthetized and transcardially perfused with cold saline and 4% paraformaldehyde until their tail and limbs were rigid on days 7, 21 and 35 post injury. The brains were removed from the skulls, fixed in 4% paraformaldehyde for seven days and embedded in paraffin. Tissues were then cut into coronal sections (4 μm thick) for immunohistochemical staining. Sections containing the hippocampal DG were incubated with the primary and secondary antibodies rabbit anti-DCX antibody (1:100) and goat anti-rabbit biotinylated antibody (1:500), respectively (both from Abcam, Cambridge, UK). DAB (Beyotime, Shanghai, China) was used as the chromogen. Positive staining of the hippocampal DG region was visualized using a microscope (Nikon, Tokyo, Japan). Images were quantitatively analyzed using IPP 6.0 software (Media Cybernetics, Inc., Rockville, MD, USA). DCX-positive cells were counted, and the mean optical density was calculated as the IOD/total area × 100.

2.9 Statistical analyses

Data were statistically analyzed using SPSS 24.0 (SPSS, Inc., Chicago, IL, USA) and are presented as the mean and standard deviation (SD). For analysis of variance (ANOVA), the homogeneity of variance was first tested by Levene's test and transformed if necessary. The BBB score, sucrose preference data (%) and serum corticosterone levels were analyzed by two-way repeated measures ANOVA. When Mauchly's test of sphericity showed $p < 0.05$, indicating that the spherical assumption had been violated, the Greenhouse-Geisser correction was applied. When the interaction effects between time and group were significant, the simple effects of time and group were analyzed and the least significant difference (LSD) was adjusted for *post hoc* comparisons. Correlations were performed using Pearson correlation analysis to assess the relationship between depressive-like behaviors and serum corticosterone levels. Differences in immobility time, Western blot data and expression levels of hippocampal DCX between groups were analyzed by one-way ANOVA with the LSD *post hoc* test. Graphs were made using Graph-

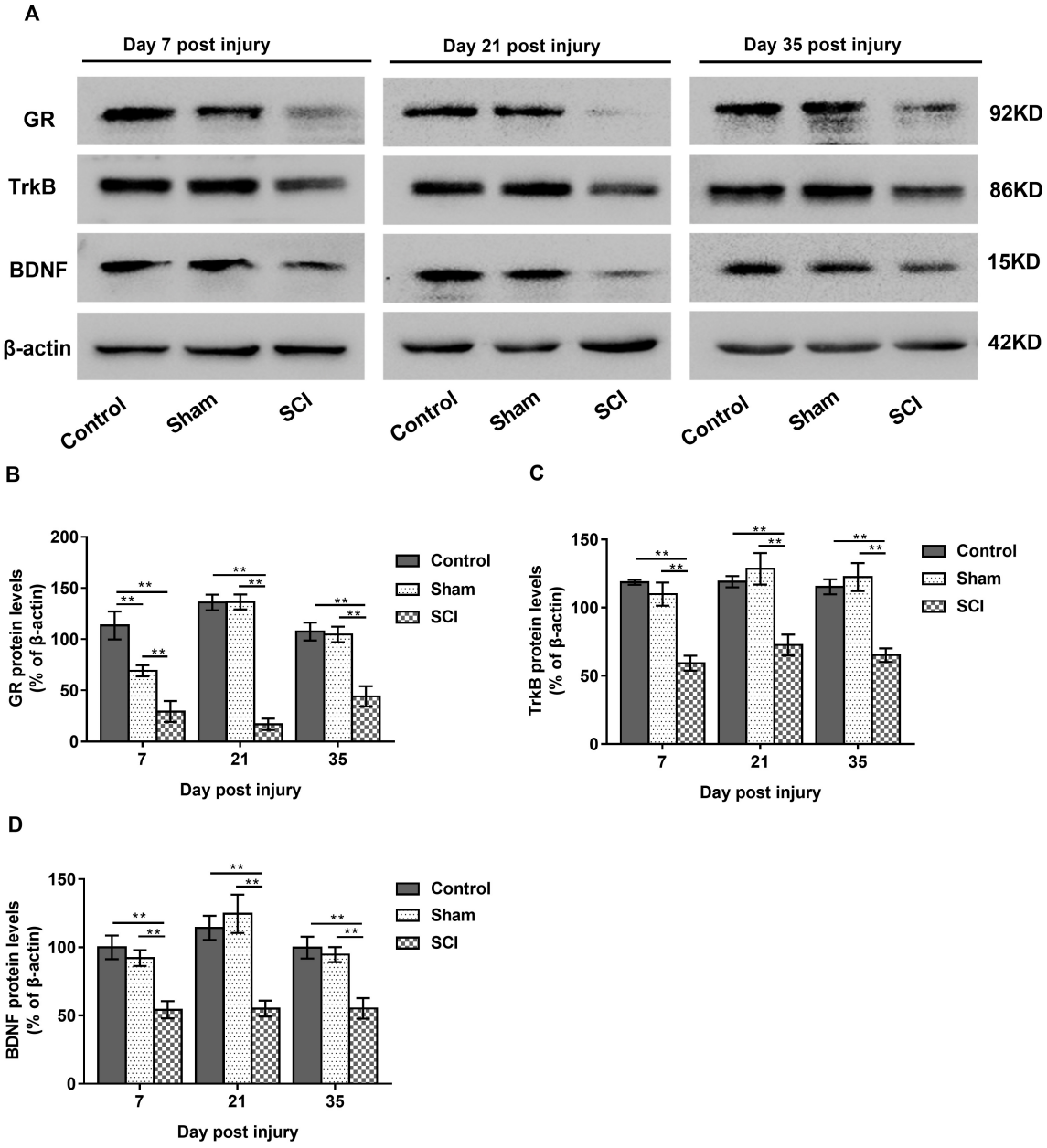

Fig. 6. Differences in group protein expression inhippocampus. Data are expressed as the mean ± SD (n = 4). (A) Representative electropherogram showing the expression of hippocampal GR, BDNF and TrkB in the control, sham and SCI groups on days 7, 21 and 35 post injury. (B–D) Protein levels in groups on days 7, 21 and 35 post injury (**$p < 0.01$).

Pad Prism 6.03 software (GraphPad Software Inc., San Diego, CA, USA). Differences for which $p < 0.05$ were considered statistically significant.

3. Results

3.1 Results of BBB score

Before injury, all rats obtained a BBB score of 21 points. As Fig. 2 shows, SCI caused motor function impairment that was not observed in the sham group or control group at any time point ($F_{5.162,54.202}$ = 115.583, $p < 0.001$). SCI rats showed a significant increase in BBB score until day 21 post injury, after which the BBB score plateaued. The BBB score of the SCI rats ranged from 2.4 ± 0.3 to 6.5 ± 0.2 points (representing extensive movement of two hindlimb joints or extensive movement of all three hindlimb joints) from day 7 to 35 post injury.

3.2 Results of depressive-like behaviors

Results showed that sucrose preference changed significantly with time ($F_{2.977,62.518}$ = 17.588, $p < 0.001$), and a significant interaction effect between time and group was observed ($F_{5.954,62.518}$ = 5.307, $p < 0.001$). As Fig. 3 shows, rats in both the SCI and sham groups exhibited decreased sucrose

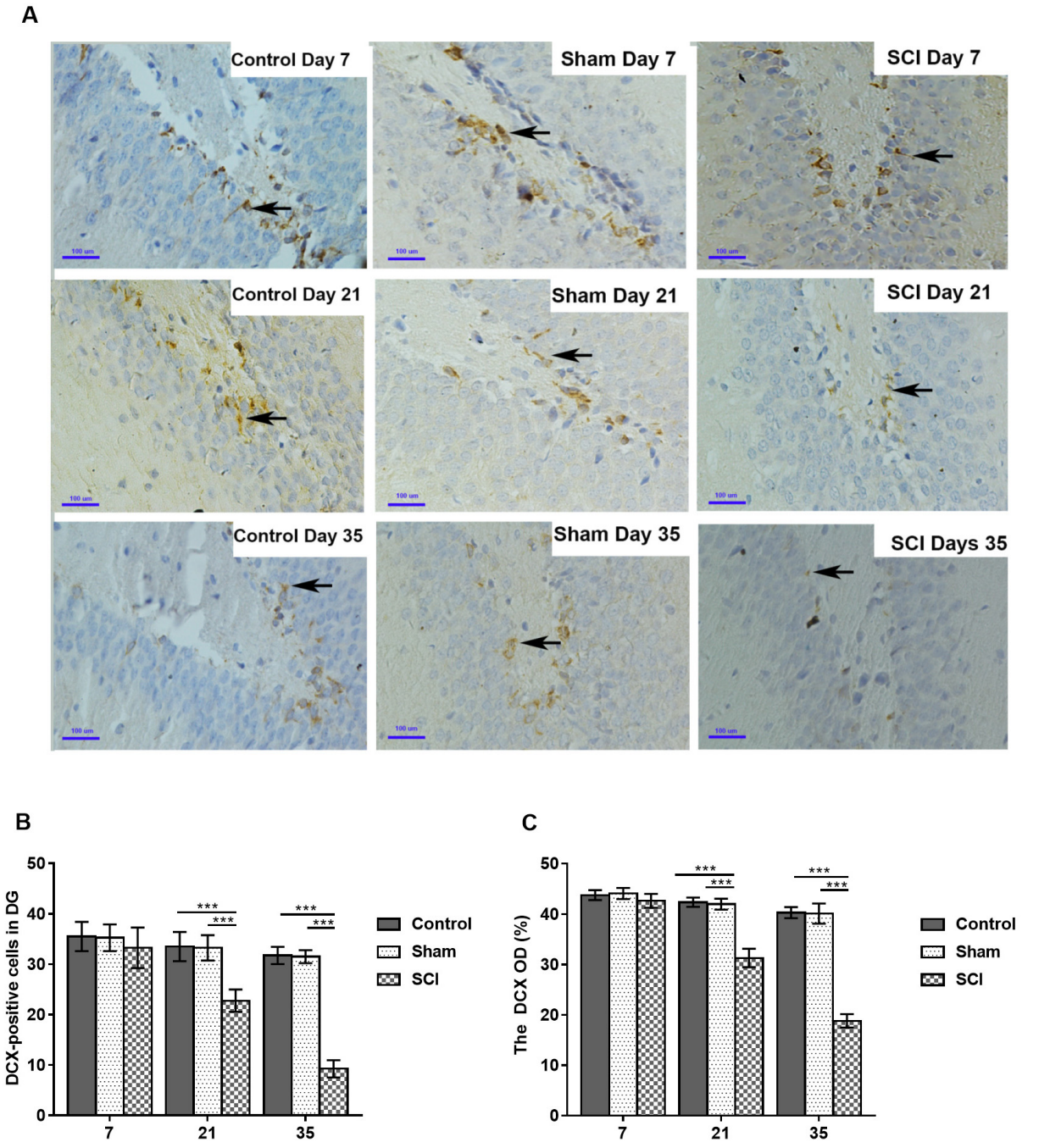

Fig. 7. DCX expression in hippocampus. Data are expressed as the mean ± SD ($n = 4$). (A) Representative images showing hippocampal DCX expression in the control, sham and SCI groups on days 7, 21 and 35 post injury; scale bars, 100 μm. Black arrows indicate DCX-positive cells (yellow). (B) The proportions of DCX-positive cells in the DG in the groups on days 7, 21 and 35 post injury. (C) DCX levels indicated by OD in the groups on days 7, 21 and 35 post injury (***$p < 0.01$; DG, dentate gyrus; OD, optical density).

preference by day six post injury and it decreased until day 12 post injury. Remarkably, sucrose preference in the SCI rats then plateaued. In contrast, sucrose preference in the sham rats gradually increased until reaching a value near normal. In the control rats, a subtle, nonsignificant decrease in sucrose preference was observed. Upon comparing that data at multiple time points, sucrose preference in the SCI group was lower than that in the control or sham group at any time point after injury. Sucrose preference in the sham group was lower than that in the control group on days 6, 12 and 19 post injury. Additionally, the SCI rats presented a significant increase in immobility time compared to those of the control and sham groups ($F_{2,21} = 4.246$, $p < 0.05$).

3.3 Results of serum corticosterone levels

As illusrated in Fig. 4, serum corticosterone levels in the sham and SCI groups were higher than baseline on day 7 post injury, after which serum corticosterone levels in the sham group decreased to baseline. Although serum corticosterone levels in the SCI group also began to decrease, they were still significantly higher than those at baseline. The serum corticosterone levels of the control rats remained stable. In addition, a significant interaction effect between time and group was observed ($F_{6,63}$ = 28.374, $P < 0.001$), and serum corticosterone levels on day 7 post injury were significantly higher in the sham and SCI groups than in the control group. Notably, serum corticosterone levels in the SCI group were significantly greater than those in the sham or control group, while serum corticosterone levels in the sham and control groups did not differ on day 21 or 35 post injury.

3.4 Correlations between serum corticosterone levels and depressive-like behaviors

Correlation analysis indicated that serum corticosterone levels were strongly negatively correlated with sucrose preference in the SCI group on days 7, 21 and 35post injury and in the sham group on day 7 post injury (Fig. 5). Moreover, serum corticosterone levels were strongly positively correlated with immobility time in the FST in the SCI group on day 35 post injury.

3.5 Results of protein expression in the hippocampus

On day seven post injury, hippocampal GR expression levels in the SCI rats were lower than those in the sham and control rats and GR protein expression in the sham rats was lower than that in the control rats ($F_{2,9}$ = 66.206, $p < 0.001$). Notably, on days 21 and 35 post injury, GR protein levels in the SCI group were still significantly lower than those in the control and sham groups ($F_{2,9}$ = 393.814, $p < 0.001$; $F_{2,9}$ = 66.164, $p < 0.001$), but there was no difference in GR protein level between the sham and control groups (Fig. 6). Additionally, SCI rats exhibited significantly lower hippocampal BDNF and TrkB protein levels than rats in the control and sham groups on day 7 ($F_{2,9}$ = 48.684, $p < 0.001$; $F_{2,9}$ = 115.660, $p < 0.001$), 21($F_{2,9}$ = 54.590, $p < 0.001$; $F_{2,9}$ = 51.327, $p < 0.001$) and 35($F_{2,9}$ = 42.404, $p < 0.001$; $F_{2,9}$ = 72.736, $p < 0.001$) post injury (Fig. 6). Moreover, compared to rats in the control and sham groups, rats in the SCI group exhibited a significantly lower number of hippocampal DCX-positive cells ($F_{2,9}$ = 23.167, $p < 0.001$; $F_{2,9}$ = 267.033, $p < 0.001$, respectively) and a significantly lower OD for hippocampal DCX ($F_{2,9}$ = 87.189, $p < 0.001$; $F_{2,9}$ = 262.253, $p < 0.001$, respectively) on day 21 and 35post injury (Fig. 7).

4. Discussion

This study aimed to illuminate the relationship between changes in depressive-like behaviors and the HPA axis in a rat model of SCI; to this end, correlation analysis was undertaken at two different time points after SCI. By monitoring from day six to thirty-five after SCI, it was found that changes in sucrose preference and immobility time were strongly correlated with serum corticosterone levels. Additionally, serum corticosterone levels increased significantly and hippocampal GR levels decreased. Furthermore, the expression levels of hippocampal DCX and BDNF/TrkB were reduced after SCI. Hyperactivation of the HPA axis and suppression of AHN may be components of the mechanism responsible for depressive-like behaviors after SCI.

The BBB score of the SCI rats on day seven post injury was 2.4 $\pm$ 0.3 (indicative of extensive movement of one or two joints), indicating that hindlimb mobility was restricted. Although motor function continued to improve more gradually, motor function recovery was limited after four weeks. The most critical results were derived from commonly used methods to assess depressive-like behaviors, such as the FST, which depends on an animal's motor function. To avoid measurement bias, an SPT that was not dependent an animal's motor function was employed to continuously monitor depressive-like behavior. Low sucrose preference indicates anhedonia, a core symptom of depression [21]. It was found that the surgical procedure resulted in anhedonia, both sham and SCI rats showed a decrease in sucrose preference until day twelve post injury. This might be due to postoperative pain. Studies have shown that lumbar laminectomy can induce postoperative pain in patients [22, 23] and thus contribute to negative emotions such as anxiety and depression [24]. Some studies have shown that patients with lumbar spine surgery whose pain level is reduced at six months following surgery were more likely to experience a reduction in depressive symptoms [25, 26]. From this, it was illustrated that the sucrose preference of rats in the Sham group in the current study returned to normal as the pain gradually relieved without any intervention. However, the state of low sucrose preference was exacerbated and prolonged in SCI rats until day 33 post injury, which was consistent with previous findings [27]. Since the study did not monitor pain after SCI, whether the changes in depressive-like behavior was caused by pain cannot be fully explained and further study may be required. Additionally, immobility in the FST as the dependent measure of behavioral despair was used to evaluate depressive-like behavior [28]. Immobility time was not consistent in the SCI rats. Findings here were similar to those of a study in which immobility time was shown to be increased in SCI rats compared to non-SCI rats [18]. However, one study found the opposite effect [27]. This discrepancy may be related to the use of different experimental designs and animal species. Therefore, the results of the behavioral tests showed that SCI caused depressive-like behaviors that persisted for a long time.

Surgery leads to an increase in serum corticosterone and cortisol levels in animals and humans, respectively [29, 30]. This study confirmed this finding, as serum corticosterone levels on day seven post injury were significantly higher in the sham and SCI groups than in the control group. Beginning on day seven post injury, the serum corticosterone level in the sham group began to decrease and returned to base-

lineby day twenty-one post injury. The levels of corticosterone in the SCI group remained high until the end of the observation period but decreased temporarily on day twenty-one post injury, essentially consistent with the change in depressive behaviors. Additionally, the serum corticosterone concentration in the SCI group was strongly correlated with depressive-like behaviors in the SPT and FST, in alignment with previous research in different chronic stress models [31–33] and research that shows that this could be reversed by body weight-supported treadmill training [34].

Further, results showed that SCI reduced hippocampal GR until day thirty-five post injury. Hippocampal GR acted as a GC receptor and the expression of hippocampal GR decreased due to a high concentration of corticosterone (animal GC) after SCI. This weakened the negative regulatory effect of the hippocampus on the HPA axis, caused hyperactivation of the HPA axis and thus exacerbation of depressive-like behaviors. The present findings were consistent with those of previous studies [7, 35] and indicated that hyperactivation of the HPA axis induced by SCI was an important neuroendocrine mechanism for changes in depressive-like behaviors.

The hippocampus is one of the main regions that receive feedback regulation from HPA axis activation [36]. Hyperactivation of the HPA axis is responsible for many of its deleterious effects on the hippocampus and behaviors [15–17]. Jure *et al.* [37] found that SCI impaired AHN on day sixty after injury. However, in this study, the expression of hippocampal DCX (as a marker for AHN) in the SCI group began to decrease on day twenty-one after injury. This may be a result of hyperactivation of the HPA axis. In turn, the inhibition of AHN weakened the negative feedback regulation of the HPA axis, further increasing serum corticosterone levels. These results are consistent with those of Snider *et al.* [38]. Masi *et al.* [15] have also shown that HPA caused the brain (particularly the hippocampus) to be exposed to corticosteroids, affecting neurobehavioral functions with an impairment of the AHN and that might be a major factor in the pathophysiology of depression. Additionally, many studies have indicated that the decreased expression of hippocampal BDNF and TrkB also inhibited AHN [39, 40]. It was therefore speculated that the decrease in hippocampal DCX expression after SCI may have been caused by downregulation of BDNF and TrkB beginning on day seven after SCI, which might simultaneously contribute to the development of depressive-like behaviors.

There were some limitations to this study. First, the number of rats tested was relatively small, which may have reduced statistical power of the analysis. Another limitation of the current work was the focus on only using DCX (a differentiated neuronal cell marker) to detect AHN, Ki67 or bromodeoxyuridine (BrdU) (proliferating cells) were not investigated here. Additionally, all analysis was correlative and it was not clear what actually caused the changes in terms of the HPA axis, AHN and depressive-like behaviors after SCI.

5. Conclusions

The results presented here suggest that SCI causes depressive-like behaviors that are associated with serum corticosterone levels, which can be used to predict depressive-like behaviors in SCI. It is suggested that hyperactivation of the HPA axis and the inhibition of AHN are part of the underlying mechanism responsible for depressive-like behaviors after SCI.

Author contributions

CHL, XHZ, ZJ performed all the experiments. CHL and BLZ contributed to the experimental design, analyzed the data, prepared figures, drafted, and wrote the manuscript. LBA and WTL revised and edited the final version of the manuscript.

Ethics approval and consent to participate

All animal procedures and care were conducted according to the institutional guidelines of the local Ethics Committee for Animal Research at Dalian University and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The Ethics Committee of the Affiliated Zhongshan Hospital of Dalian University approved the study (reference number, 2017082).

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Conflict of interest

The authors declare no conflict of interest.

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