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Neuroplastic and Neuroinflammatory Alterations in the Hippocampus after Spinal Cord Injury

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Abstract—Objective: Spinal cord injury (SCI) is a debilitating condition that not only affects motor and sensory functions, but also causes widespread neuroplastic and neuroinflammatory changes in the hippocampus, a brain region critical for learning and memory. **Methods:** This study examined region-specific changes in hippocampal subregions (CA1, CA3, and dentate gyrus) in a rat model of SCI, evaluating neurogenesis, microglial and astroglial cell activation, and behavioral outcomes. **Results and Discussion:** The study revealed distinct patterns of neuroplastic and neuroinflammatory changes in hippocampal subregions, with some areas showing increased susceptibility to injury-induced changes. Behavioral analyses showed a significant correlation between these hippocampal changes and impairments in memory and learning ability, highlighting the functional consequences of SCI on cognitive processes. **Conclusions:** The findings emphasize the importance of understanding the relationship between neuroplasticity and neuroinflammation in the hippocampus after SCI, which may facilitate the development of targeted therapeutic strategies to mitigate cognitive deficits.

Keywords*:* spinal cord injury, neuroinflammation, hippocampus, memory, rats

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INTRODUCTION

Spinal cord injury (SCI) is one of the most severe and disabling conditions with a multifaceted impact on the patient's body. In addition to the obvious motor and sensory impairments, SCI causes profound changes in the central nervous system (CNS), affecting both the spinal cord and brain (Wu et al., 2024). One of the core structures affected by SCI is the hippocampus, a region of the brain critical for learning, memory, and emotional regulation processes (Kalkhoran et al., 2022). Research in recent years suggests that spinal cord injury triggers a cascade of neuroplastic and neuroinflammatory processes in the hippocampus that may have long-term consequences for the cognitive status of patients (Sefiani and Geoffroy, 2021; Ma et al., 2024).

The hippocampus, due to its unique structure, plays a central role in memory formation and consolidation (Nadel et al., 2007; Anand and Dhikav, 2012) as well as in spatial navigation (Eichenbaum, 2017), while its high plasticity makes it vulnerable to a variety of pathological influences, including the following a SCI event (Sefiani and Geoffroy, 2021; Marzola et al., 2023; Ma et al., 2024). Neuroplastic alterations, such as impairment of neurogenesis as well as synaptic plasticity, are observed already in the first days after injury (Marzola et al., 2023) and are accompanied by a strong neuroinflammatory response characterized by the activation of both microglia and astroglia, which

normally perform protective functions but can contribute to the development of a chronic inflammatory state upon overactivation (Wu et al., 2014).

The region-specific nature of changes in the hippocampus after SCI is of special concern (Jure et al., 2022). Studies conducted on animal models, particularly in rats, have shown that different subregions of the hippocampus, such as CA1, CA3, and dentate gyrus, exhibit different patterns of neuroplastic and neuroinflammatory changes. For example, the CA1 region shows a significant decrease in neuronal density, whereas the dentate gyrus shows impaired neurogenesis (Sefiani and Geoffroy, 2021). One of the key mechanisms of this disorder is neuroinflammation, which is the immune system's response to injury, accompanied by activation of microglia, astrocytes, and release of proinflammatory cytokines (IL-1β, TNF-α, IL-6) (Adamu et al., 2024). Studies show that as early as the first days after injury, levels of pro-inflammatory molecules are increased in the hippocampus, leading to inhibition of neural stem cell proliferation and reduced survival of new neurons (Zangbar et al., 2021; Jure et al., 2022; Kalkhoran et al., 2022).

Evidence from behavioral experiments in animal models confirms that changes in the hippocampus after SCI have functional consequences. Impairments in spatial and contextual memory, as well as reduced learning ability, directly correlate with neuroplastic and neuroinflammatory changes in the hippocampus (Kalkhoran et al., 2022). These findings emphasize that SCI not only limits motor activity but also has profound effects on cognitive function (Alcántar-Garibay et al., 2022). Understanding the mechanisms underlying neuroplasticity and neuroinflammation in the hippocampus after SCI is important for the development of new therapeutic strategies. Current approaches, such as modulation of neuroinflammation (Lee et al., 2022), stimulation of neurogenesis (Balseanu et al., 2020), and administration of neuroprotective drugs (Zhang et al., 2021), may help minimize cognitive impairment and improve the quality of life of SCI patients. However, for these approaches to be successful, further research is needed to investigate region-specific changes in the hippocampus and their functional consequences.

The purpose of the present work was to analyze neuroplastic and neuroinflammatory changes in the hippocampus of rats 4 weeks after spinal cord injury. Immunohistochemical evaluation was performed in CA1 and CA3 regions of the hippocampus, as well as in the dentate gyrus (DG) area. The cognitive function of the animals was assessed in the Novel Object Recognition test (NOR).

MATERIALS AND METHODS

Animals

The study was performed on 20 male Wistar rats aged 3 months and weighing 250–300 g. The animals were obtained from Rappolovo laboratory animal nursery (Leningrad region, Russia). Rats were kept 5 individuals per cage at room temperature 20–22°C and light regime 12 h light 750 lx/12 h darkness. All animals received a standard food ration (dry complete granulated extruded compound feed, recipe PK-120, LLC "LABORATORKORM," Russia) and had free access to food and water. The rats were quarantined for 14 days before starting the experiments. All animals were divided into two groups: group SHAM, false-operated animals; and group SCI, animals with left-sided hemisection of the spinal cord.

SCI Modeling

To perform lateral left-sided hemisection of the spinal cord, rats were anesthetized by intramuscular injection of combination of zolazepam/tiletamine (Zoletil 100, 60 mg/kg) and xilazine (Xyla, 2 mg/kg; Interchemie, Netherlands). A midline skin incision was then made in the area between and caudal to the scapulae, bluntly dividing the muscles of the vertebral column to allow access to the spine. The T7 and T9 vertebrae were cleared from soft tissue and muscle from the lateral sides and fixed with vertebral clips in spinal stereotaxis, so that the spinal cord was strictly horizontal. After resection of the arch of the 8th thoracic vertebra was performed, a metal guide made from half of a 21G needle was vertically inserted into the spinal cord along its midline. A single-use microscalpel was inserted centrally into the spinal cord using a guide, and then a lateral incision was made. Minor bleeding after transection was stopped with a cotton swab applied for 1–2 min. The back muscles were then sutured with continuous sutures using Vicryl 4 absorbable suture material and the skin was sutured with interrupted sutures using Ethilon 4 non-absorbable suture material.

The study also included the "SHAM" group that underwent a laminectomy without spinal cord trauma

to control the effect of surgical intervention on the experimental results. All surgical manipulations were performed under aseptic conditions; instruments were sterilized in a dry heat sterilizer beforehand. After the surgery, the animals were placed in clean cages and monitored until complete recovery from anesthesia. Analgesic (ketorolac, 1 mg/kg, s/c) was administered every 12 h for 48 h to relieve postoperative pain. Antibiotic (enrofloxacin, 5 mg/kg, s/c) was administered daily for 7 days to prevent urinary tract infection. In the first days after surgery, water intake was monitored and, if necessary, injections of saline solution in the amount of 3–5 mL subcutaneously were performed. The bladder was emptied manually once a day until voluntary urination was restored.

Novel Object Recognition Test

In the present study, cognitive functions of rats were assessed using the NOR test, a classical behavioral test based on the natural tendency of rodents to show increased interest in unfamiliar objects (Mathiasen and DiCamillo, 2010). This method allows quantitative assessment of long-term memory through the calculation of the discrimination index (the ratio of the time spent exploring a new object to the total time spent exploring all objects). Animals were tested one week before the operation and four weeks after, a few days before the animals were removed from the experiment. The habituation phase was conducted one day before the familiarization session with the objects. Each animal was placed in a circular arena (97 cm diameter) without objects and tested for 5 min. The next day, each rat was placed in the same arena containing two identical plastic objects on the left and right sides of the arena for 5 min during the familiarization phase. The animal was then returned to the original cage for a holding period of 24 h (to allow testing of long-term memory). Each rat was placed in a test arena where one of the objects was replaced with a new one. Both objects were placed at the same distance from the animal. A recording device was placed over the test setup to continuously videorecord the behavior. The animal's interest in the object was determined by positioning its nose at a distance of no more than 2 cm from the object. Animal tracking was performed using Noldus EthoVision HT software (Noldus; Wageningen, Netherlands). The discrimination index was calculated as the ratio of the time spent exploring the new object to the total time spent exploring both objects. After each animal, the objects and arena were thoroughly cleaned with 10% ethanol.

BrdU Labelling

BrdU, a thymidine analog that is incorporated into synthesized DNA, was used in the study. Administration of the drug (50 mg/kg, 0.008 N NaOH) was performed intraperitoneally for 3 postoperative days.

Fixation and Tissue Processing

A few days after behavioral testing, rats were anesthetized with an overdose of Zoletil 100 (Virbac, France) and xylazine (Interchemie werken "De Adelaar," Netherlands) and transcardially perfused with 200 mL of ice-cold saline (4°C) followed by 200 mL of cold fixative (4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2). The brain was immediately extracted and postfixed for 12 h at 4°C in freshly buffered 4% paraformaldehyde. Then the brain samples were washed in a phosphate-salt buffer (PBS). The olfactory bulbs and cerebellum were removed using a microtome blade. Next, coronal sections of the brain were made on a vibrating blade microtome (Leica VT1000S; Leica Biosystems, USA). The prepared brain was fixed on the holder using cyanoacrylate adhesive. The blade was positioned at an angle of 10°. The following cutting parameters were used: cutting speed 7.0, maximum cutting frequency, and slice thickness 50 μm. Ready slices were further stored in a cryoprotective solution based on ethylene glycol and glycerol at –20°C.

Immunohistochemical Study of Glial Cells

For immunohistochemical staining, dorsal hippocampal slices, selected at a standardized level relative to bregma, were washed sequentially with PBS-T solution (0.1% Tween-20 in phosphate-salt buffer). Immunohistochemical staining with antibodies to Iba-1 (ionized calcium-binding adapter molecule 1), a specific marker of microglial cells, was performed to comprehensively analyze the state of microglia in the hippocampus. Iba-1 is a calcium-binding adapter protein with a molecular mass of 17 kDa, which is expressed exclusively in microglia cells and plays a key role in the processes of membrane remodeling and phagocytosis. The methodological advantages of this marker include stable expression under various pathological conditions, clear visualization of cellular outgrowths, and the possibility of accurate quantification. Staining for glial fibrillary acidic protein (GFAP), a specific marker of CNS

astrocytes belonging to proteins of class III of intermediate filaments, in combination with labeling of microglia using antibodies to Iba-1, allows a comprehensive analysis of neuroglial interactions and a differentiated assessment of the contribution of astrocytic and microglial components to the development of pathological changes in the model of spinal cord injury.

To suppress endogenous peroxidase activity, slices were incubated in 3% H_2O_2 solution for 15 min at room temperature. Blocking of nonspecific binding was then performed by one-hour incubation in PBS-based blocking buffer containing bovine serum albumin (BSA) and 0.3% Triton X-100. Mature astrocytes were stained with mouse anti-GFAP primary antibody at a dilution of 1 : 2000 (#14-9892-82; Thermo Fisher Scientific, USA), and microglial cells were stained with rabbit polyclonal anti-Iba-1 antibody at a dilution of 1 : 1000 (# 019-19741; FUJIFILM Wako Pure Chemical Corporation, Japan). Free-floating slices were incubated at 4°C in the antibody solution for 12 h. The next day, slices were washed with cold PBS-T solution. For secondary signal detection we used a commercial kit with antibodies to mouse/rabbit IgG conjugated to polymeric horseradish peroxidase (poly-HRP) (no. 78-310004-15; Primebiomed, Russia), staining was performed strictly according to the manufacturer's instructions. After staining the sections were washed in a phosphate buffer, mounted on object slides, dehydrated in isopropanol, clarified in xylene and coverslipped with Vitrogel mounting medium (ErgoProduction, Russia).

Staining of BrdU Labelled Cells

Immunohistochemical staining of BrdU-positive cells in the subgranular zone of the dentate gyrus was performed using primary antibodies to BrdU at a dilution of 1 : 2000 (#555627; BD Pharmingen, USA) according to a similar protocol except for additional steps: denaturation of DNA in 2 N HCl at 37°C for 45 min and subsequent neutralization of the acid in 0.1 M borate buffer (pH 8.5, 30 min).

Image Processing and Analysis

Images were acquired on a ZeissAxioScope A1 microscope with a 20×/0.45 objective lens equipped with a ZEISS Axiocam 503 color camera, and micrographs of AxiosVision software (Zeiss, Germany) were saved as TIFF files. ImageJ program (NIH, Bethesda, MD, USA) was used for image processing and analysis. Counting was performed in separate layers of each hippocampal zone (CA1, CA3, DG). After converting images to 8-bit format, background subtraction and contrast enhancement, areas of specific staining were highlighted using the Threshold tool (Image > Adjust > Threshold). The percentage of stained area was calculated as the ratio of the area of positive signal to the total area of the analyzed region. BrdU-positive cells were counted in the subgranular region of the dentate gyrus of the hippocampus. For spatial standardization, pixel values were converted to μm^2. Cell density was calculated with tissue volume, using a conversion factor to convert to mm^3 values. At least 40 images of the ipsi- and contralateral hippocampus for each group of animals were used for analysis. Images were imported into the Figma design platform (United States, 2023, Version 116.5) and were brightness and contrast corrected to optimize clarity.

Statistical Analysis

All data are presented as mean ± standard deviation (SD). Significant differences were defined as (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$, and (****) $p < 0.0001$. To evaluate the influence of multiple independent factors on the studied parameters, two-factor analysis of variance with repeated measures (two-way mixed ANOVA) was applied. For pairwise comparisons between two independent groups when data violated assumptions of normality or homogeneity of variances, multiple Mann–Whitney U tests were conducted with Bonferroni adjustment to control for family-wise error rate. Experiments involving quantitative analysis of immunohistochemical staining were performed with randomization of groups and adherence to the blinding principle at all stages of the study. Microsoft Excel software (Microsoft; Oklahoma, USA) and GraphPad Prism 10 (GraphPad Software; California, USA) were used for statistical analysis.

RESULTS

The Effect of Spinal Cord Injury on Long-Term Memory

Before surgical intervention, both groups showed comparable performance in the Novel Object Recognition (NOR) test, as measured by the discrimination index (DI): "SHAM" group—0.37 ± 0.17, "SCI" group—0.40 ± 0.15 ($p > 0.05$). However, significant differences between the groups were noted postoperatively. In the non-injured group, the values of DI before surgical intervention, both

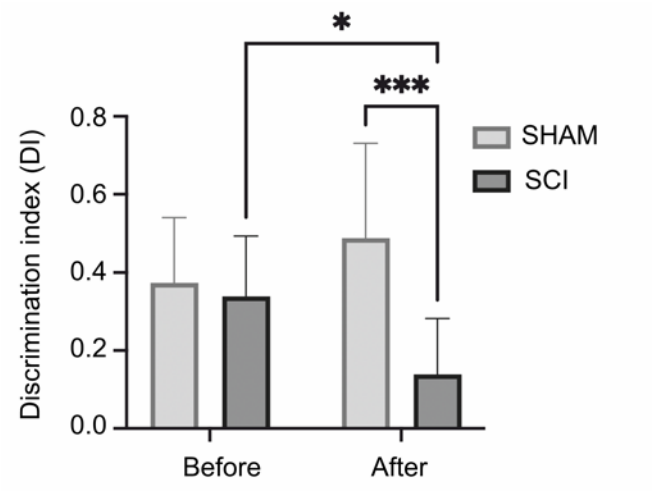

Fig. 1. Comparison of Novel object recognition test in SHAM and SCI rats before and after surgery. Data are presented as mean ± SD, (***) $p < 0.001$, (*) $p < 0.05$.

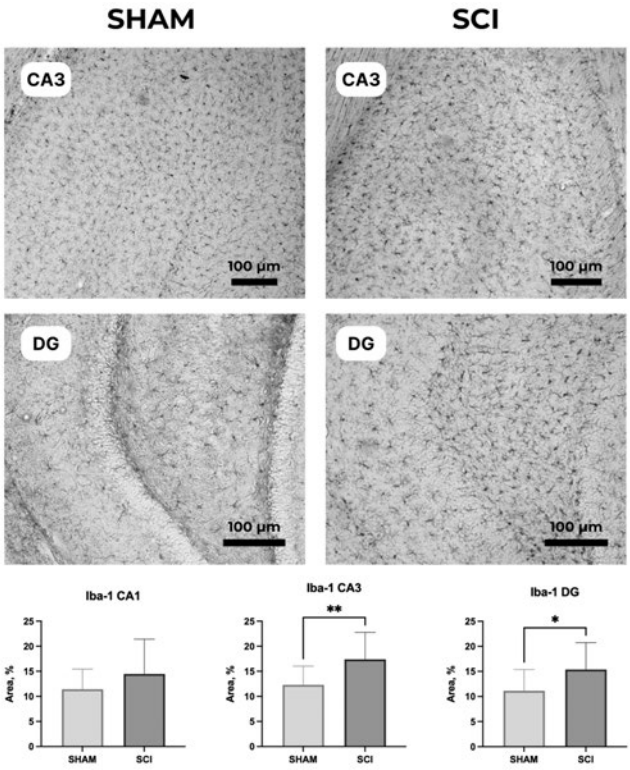

Fig. 2. Examples of representative images obtained by immunohistochemical staining of the hippocampus for the microglia marker Iba-1. Comparison of Iba-1-positive cell density in hippocampal regions (CA1, CA3, DG) between SHAM and SCI rats, mean ± SD, (**) $p < 0.01$, (*) $p < 0.05$. Scale bar: 100 µm.

groups showed comparable performance in the NOR test: "SHAM" group—0.37 ± 0.17, "SCI" group—0.40 ± 0.15 ($p > 0.05$). However, significant differences between the groups were noted postoperatively. In the non-injured group, the values remained stable (0.49 ± 0.24), whereas animals with lateral hemisection showed a marked decrease to 0.14 ± 0.14 four weeks post-injury ($p < 0.05$ compared to baseline values and $p < 0.001$ vs SHAM group). Data are presented in Fig. 1.

Effect of Spinal Cord Injury on the Expression of Micro- and Astroglial Markers in the Hippocampus

In the present study, micrographs of slices with immunohistochemical staining for major micro- and astroglial markers were analyzed. Immunohistochemical study with Iba-1 marker revealed significant regional differences in microglia activation in the hippocampus after spinal cord injury at $p < 0.05$. The most pronounced changes were observed in the CA3 area, where there was a significant increase in the number of Iba-1-positive cells compared to the control group (SHAM: 12.30 ± 3.73% vs SCI: 17.38 ± 5.38%; $p < 0.01$). Microglial cells in this area showed characteristic signs of activation: increase in the size of cell bodies, thickening and shortening of branches, as well as changes in their spatial distribution. In contrast to the CA3 zone, no statistically significant increase in the density of Iba-1-positive cells was found in the CA1 region (SHAM: 11.42 ± 4.03% vs SCI: 11.47 ± 6.92%; $p > 0.05$). Microglia in this region retained a predominantly quiescent morphology with thin, well branched outgrowths, suggesting selective vulnerability of different hippocampal regions to the systemic effects of spinal injury. Notably, significant changes were observed in the dentate gyrus, where a significant increase in the number of activated microglial cells was also recorded (SHAM: 11.14 ± 4.24% vs SCI: 15.39 ± 5.34%; $p < 0.05$). Data and representative photographs of staining are shown in Fig. 2.

Immunohistochemical analysis of glial fibrillary acidic protein (GFAP) distribution in the hippocampus revealed significant intergroup differences between animals with spinal cord injury (SCI) and controls (SHAM). The CA areas showed a pronounced layer-specific astrocyte response. In the CA1 area, the SCI group showed a significant increase in GFAP positivity in the stratum oriens (SCI: 2.80 ± 0.74% vs SHAM: 2.21 ± 0.49%; $p < 0.05$) and stratum radiatum (SCI: 4.81 ± 0.60% vs SHAM: 3.52 ± 0.68 %; $p < 0.05$), whereas no differences

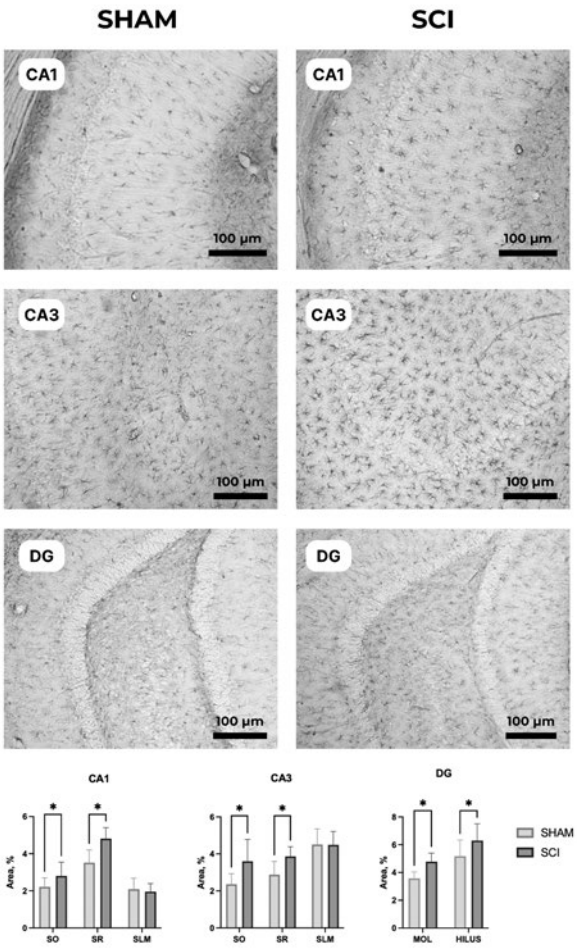

Fig. 3. Spinal cord injury effects on astroglia activity in the hippocampus. Examples of representative images obtained by immunohistochemical staining of the hippocampus for the astroglia marker GFAP. SO, stratum oriens; SR, stratum radiatum; SLM, stratum lacunosum-moleculare B. Percentage of area covered by GFAP immunopositive staining of hippocampal areas 4 weeks after surgery, mean ± SD, (*) $p < 0.05$. Scale bar: 100 μm.

were found in stratum lacunosum-moleculare (SLM). A similar pattern was observed in CA3 region with increased GFAP expression in stratum oriens (SCI: 3.60 ± 1.18% vs SHAM: 2.36 ± 0.57%) and stratum radiatum (SCI: 3.87 ± 0.52% vs SHAM: 2.88 ± 0.72%) with no significant changes in SLM. Of particular interest were the changes in the dentate gyrus, where a complex pattern of reactive gliosis was detected. In the molecular layer, an increase in the density of $GFAP^+$ cells with a characteristic change in the morphology of astrocytes by 1.3% points was recorded ($p < 0.05$). Expressed changes were found in the hilus, where a marked elevation in GFAP-positive staining by 1.2% points ($p < 0.05$) with the formation of focal clusters of hypertrophied astrocytes and increased glial fibrosis in the perivascular areas was observed. Data and representative photographs of staining are shown in Fig. 3.

Effect of Spinal Cord Injury on Hippocampal Neurogenesis

In the present study, neurogenesis in the hippocampus after SCI was analyzed using BrdU (bromodeoxyuridine) immunohistochemical labelling specific for proliferating cells. The number of BrdU-positive cells was evaluated in two groups: SHAM and SCI. Statistical analysis (Mann–Whitney test) revealed a significant decrease in the number of BrdU-positive cells in the hippocampus after SCI ($p < 0.001$). The mean number of cells was 55173 ± 18808 cells/mm^3 (SHAM) vs 35014 ± 22781 cells/mm^3 (SCI). These findings suggest that under experimental conditions SCI causes a decrease in neurogenesis in the hippocampus. Quantitative data and representative photographs of staining are presented in Fig. 4.

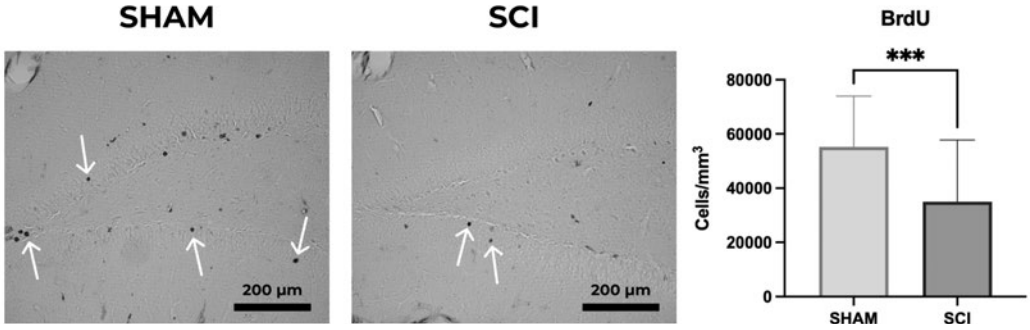

Fig. 4. Effect of spinal cord injury on hippocampal neurogenesis. Representative microphotographs of BrdU immunostaining in SHAM and SCI groups. Quantitative analysis of BrdU-positive cells in the hippocampus, data are present as mean ± SD, (***) $p < 0.001$. Scale bar: 200 μm.

DISCUSSION

Spinal cord injury (SCI) remains a serious medical and social problem with the highest prevalence in the young population (Ding et al., 2022). The socioeconomic burden of SCI is extremely high due to the need for long-term treatment, rehabilitation and ongoing care, and the consequences of the injury are highly variable and depend on the level and extent of injury. A special place among secondary posttrauma consequences is occupied by neuropathic pain, manifested by allodynia and hyperalgesia, which significantly worsens the quality of life of patients (Forte et al., 2022), as well as psychological consequences (depression, anxiety disorders, adaptation disorders) and cognitive impairment, especially in patients with a high level of lesions or chronic pain (Budd et al., 2022). These complications form a challenging picture of the consequences of trauma and require a complex interdisciplinary approach to treatment and rehabilitation. However, even modern therapies are mainly aimed only at symptom management without addressing the underlying causes of functional disorders. Numerous studies provide evidence that spinal cord injury induces a generalized neuroinflammatory process in the brain (Maldonado-Bouchard et al., 2016; Jure et al., 2017). It has been shown that ischemic neuroinflammation leads to structural and functional changes in subcortical structures, including the limbic system and basal ganglia, which in turn may explain the persistence of neuropathic pain and the development of associated psychoemotional disorders in patients, such as depression and anxiety (Wang et al., 2022). A special role in these processes is played by the hippocampus, a complexly organized structure consisting of several clearly differentiated layers, each of which performs specific functions in learning and memory processes (Leutgeb et al., 2007; Schultz and Engelhardt, 2014). The high sensitivity to neuroinflammatory changes makes it a key target for pathologic conditions associated with spinal cord injury.

The results obtained in the present study demonstrate a pronounced regional specificity of microglia and astroglia activation in the hippocampus after spinal cord injury. The most significant changes in microglia distribution were observed in the hippocampal CA3 subregion, where the number of Iba-1-positive cells with characteristic morphological signs of cell activation increased significantly. These data indicate the development of neuroinflammatory response in this particular hippocampal area, which may be related to the peculiarities of its neuronal organization—the presence of recurrent collaterals and high plasticity of synaptic connections. In contrast to CA3, the CA1 area remained relatively intact, which was manifested by the absence of statistically significant changes in microglia density and the predominance of cells with quiescent morphology. Of particular interest is the response of the dentate gyrus, where we also observed a significant increase in the number of activated microglial cells, which may have important implications for neurogenesis and integration of new neurons. Our findings of region-specific activation of microglia in the hippocampus after spinal cord injury are consistent with current ideas about the heterogeneity of microglial responses in different brain structures (Tan et al., 2020). The relative preservation of the CA1 region observed in our study contradicts some findings of uniform activation of microglia in all hippocampal regions after neural damage (Tyrtyshnaia et al., 2019; Jure et al., 2022). However, our results are consistent with other work showing a selective vulnerability of the CA3 region as a result of compression spinal cord injury of various severity (Jure et al., 2017). Studies suggest that the increased sensitivity of the CA3 region to systemic inflammatory signals may be due to the constant receipt of massive innervation from the entorhinal cortex to this region, which increases its susceptibility to inflammatory influences (Hernández-Frausto and Vivar, 2024), and may also be related to the peculiarities of the local organization of neurons with a predominance of collateral connections, which may create an increased vulnerability of synaptic transmission during the development of systemic inflammation (Leutgeb et al., 2007). This may be due to differences in chemokine receptor expression, as CA3 neurons have been shown to express more C–C chemokine receptor type 2 (CCR2, mediate agonist-dependent calcium mobilization and inhibition of adenylate cyclase), making them more sensitive to pro-inflammatory signals (Knerlich-Lukoschus et al., 2011). In addition to microglial activation, in the present study immunohistochemical analysis showed a significant increase in astroglial marker expression in both CA1 and CA3 in the stratum oriens layer, which contains basal dendrites of pyramidal neurons and Schaffer collaterals (playing a key role in the formation of associative connections), as well as in the stratum pyramidale layer, where the main excitatory neurons of the hippocampus are localized. In the dentate gyrus, particularly expressed GFAP-positivity is noted in the molecular layer and hilus, where astrocytes form dense perineuronal and

perivascular structures. In the molecular layer, dense perineuronal glial structures provide metabolic support for synaptic transmission and regulate neuronal activity, particularly important for processing afferent inputs from the entorhinal cortex. Simultaneously, the pronounced astroglial response in the hilus, where mossy fibers, interneurons, and vascular plexuses are concentrated, indicates the participation of astrocytes in the regulation of neuronal circuits, control of the blood-brain barrier, and neurovascular interaction. Such spatially specific activation of astrocytes may represent both an adaptive response aimed at maintaining neuronal homeostasis and a potential pathological component contributing to the formation of abnormal neuroglial networks. These findings are consistent with current ideas about the critical role of astrocytes in the regulation of dentate gyrus neuronal networks (Sierra et al., 2014) and their contribution to the pathogenesis of neurodegenerative processes (Jure et al., 2017). The revealed moderate increase in the density of GFAP-positive cells in different regions of the hippocampus, despite its relatively minor magnitude, may reflect functionally significant changes in reactive astrogliosis, which is consistent with the data of modern studies demonstrating the biological significance of various astroglial responses, including minimal reactions in various CNS disorders and injuries (Escartin et al., 2021). At the same time, the analysis of the dynamics of glial cell activation indicates a probable temporal continuity in their response, where the primary activation of microglia acts as a key trigger of the neuroinflammatory cascade, while the secondary reaction of astrocytes develops later and is accompanied by a characteristic morphological transformation, indicating the progression of the reactive state (Liddelow et al., 2017; Liu et al., 2020). In addition, the results obtained in the present work also demonstrate a significant decrease in the number of BrdU-positive cells in the dentate gyrus of the hippocampus in animals with spinal cord injury compared to controls. These data are consistent with numerous studies (Sefiani and Geoffroy, 2021; Ma et al., 2024), which also observed inhibition of neurogenesis in the hippocampus after spinal injury and associated such changes with many factors of neuroinflammatory processes, but primarily with the activation of both astro- and microglia.

Of particular significance is the relationship found between astrocytic and microglial responses and cognitive deficits. The impairment of long-term memory in recognition tests can be explained by several mechanisms: impaired synaptic plasticity due to changes in neuroglial interactions, dysfunction of neuronal networks due to modification of the extracellular matrix, and impaired neurogenesis in the dentate gyrus. The coincidence of zones of the most pronounced glial activation (CA3 and dentate gyrus) with areas critical for learning and memory processes is particularly indicative. The revealed selective vulnerability of different hippocampal regions can be explained by differences in their afferent innervation and different expression of receptors to proinflammatory mediators; however, further studies are needed to clarify the molecular mechanisms of glial activation and its impact on neurogenesis, synaptic plasticity, and cognitive function.

CONCLUSION

This study demonstrates that spinal cord injury causes significant impairment of long-term memory and simultaneous activation of microglia and astrocytes in the hippocampus with regional differences (most pronounced in the CA3 area and dentate gyrus), as well as decreased neurogenesis, which indicates a complex effect of SCI on cognitive functions and neuroplasticity. The findings emphasize the importance of further study of the mechanisms of neurodegenerative changes after SCI to develop strategies for neuroprotection and restoration of cognitive functions.

ABBREVIATIONS AND NOTATION

BrdU—bromodeoxyuridine;

CCR2—C-C chemokine receptor type 2;

CNS—central nervous system;

DG—dentate gyrus area;

GFAP—glial fibrillary acidic protein;

Iba-1—Ionized calcium binding adaptor molecule 1;

NOR—novel object recognition test;

s/c—subcutaneously;

SCI—spinal cord injury.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Animal-related experiments were conducted in accordance with the NIH Guidelines for the care and use of laboratory animals (http://oacu.od.nih.gov/regs/index.htm). Animal protocol was approved by the Ethics Committee of St. Petersburg State Chemical and Pharmaceutical University of the Ministry of Health of Russia (protocol application: R-SCI1-SA2023 dated February 15, 2023). All measures were taken to reduce the number of animals used and minimize their suffering.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

AUTHOR CONTRIBUTION

Conceptualization—YuIS, PEM, MYS; Methodology—SPK, PYuS, YuIS, AV; Formal analysis and investigation—SPK, AV, VSS, KAA; Writing, original draft preparation—SPK, YuIS, VSS, KAA; Writing, review and editing—SPK, YuIS, PEM; Funding acquisition—PEM; Resources—YuIS, SVO, MYS, PEM; Supervision—PEM. All authors have read and agreed to the published version of the manuscript.

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AI tools may have been used in the translation or editing of this article.