

Synaptosomal-associated protein 25 may be an intervention target for improving sensory and locomotor functions after spinal cord contusion

Zhan-qiong Zhong^{1,2}, Yang Xiang¹, Xi Hu¹, You-cui Wang³, Xi Zeng¹, Xiao-meng Wang¹, Qing-jie Xia³, Ting-hua Wang³, Xiao Zhang^{1,*}

1 Experiment Technology Center of Preclinical Medicine of Chengdu Medical College, Chengdu, Sichuan Province, China

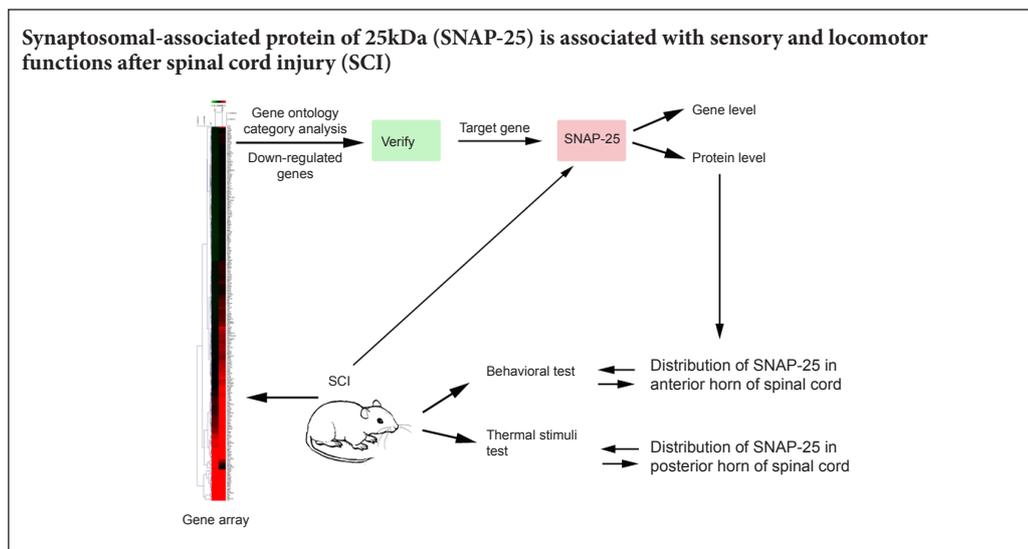
2 School of Basic Medicine, Chengdu University of Traditional Chinese Medicine, Chengdu, Sichuan Province, China

3 Institute of Neurological Diseases, Center for Translational Neuroscience, Western China Hospital, Sichuan University, Chengdu, Sichuan Province, China

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Graphical Abstract



*Correspondence to:

Xiao Zhang, Ph.D.,
zhangxiao1985007@126.com.

orcid:

0000-0001-6597-8825
(Xiao Zhang)

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Abstract

Synaptosomal-associated protein 25 kDa (SNAP-25) is localized on the synapse and participates in exocytosis and neurotransmitter release. Decreased expression of SNAP-25 is associated with Alzheimer's disease and attention deficit/hyperactivity disorder. However, the expression of SNAP-25 in spinal cord contusion injury is still unclear. We hypothesized that SNAP-25 is associated with sensory and locomotor functions after spinal cord injury. We established rat models of spinal cord contusion injury to detect gene changes with a gene array. A decreased level of SNAP-25 was detected by quantitative real time-polymerase chain reaction and western blot assay at 1, 3, 7, 14 and 28 days post injury. SNAP-25 was localized in the cytoplasm of neurons of the anterior and posterior horns, which are involved in locomotor and sensory functions. Our data suggest that reduced levels of SNAP-25 are associated with sensory and locomotor functions in rats with spinal cord contusion injury.

Key Words: nerve regeneration; synaptosomal-associated protein 25 kDa; sensory function; locomotor function; spinal cord injury; gene array; neurons; neural regeneration

Introduction

Spinal cord injury (SCI) is a traumatic injury that leads to devastating motor and sensory dysfunction. The primary and complex secondary injuries, including cell apoptosis and axonal degeneration, both contribute to the dysfunction after SCI (Ahuja and Fehlings, 2016). Identification of key molecules in the mechanism of SCI-induced dysfunction could lead to effective ways to ameliorate the loss of motor

and sensory functions (Gensel and Zhang, 2015).

Synaptosomal-associated protein 25 kDa (SNAP-25) is a member of a family of evolutionarily conserved proteins and is essential for neurotransmitter release. SNAP-25, syntaxin-1 and synaptobrevin form the stable soluble N-ethylmaleimide sensitive factor attachment protein receptor complex, and their interaction is involved in the release of synaptic vesicles (Jahn et al., 2003; Jahn and Scheller, 2006).

SNAP-25 is located on the synapse and is involved in rapid and slow endocytosis of secretory vesicles (Xu et al., 2013). In neuroendocrine cells, SNAP-25 is localized to the plasma membrane where it functions in regulated secretory vesicle exocytosis, but it is also found on intracellular membranes (Aikawa et al., 2006). In neonatal deep cerebellar nuclei, SNAP-25 is detectable in both vesicular glutamate transporter 1- and 2-immunoreactive synaptic contacts, with a higher prevalence in vesicular glutamate transporter 1-positive terminals (Manca et al., 2014). The distribution and the role of SNAP-25 in spinal cord are still unclear.

Other studies have recently reported the relationship of SNAP-25 with Alzheimer's disease (Brinkmalm et al., 2014), attention deficit/hyperactivity disorder (Galvez et al., 2014), and autism (Braida et al., 2015). Increased levels of SNAP-25 were found in Alzheimer's disease patients, in whom cerebrospinal fluid levels of SNAP-25 could serve as a marker

for the diagnosis of Alzheimer's disease (Brinkmalm et al., 2014; Guerini et al., 2014). Barakauskas et al. (2016) have shown that lower levels of SNAP-25 in schizophrenia may be associated with greater illness severity, and polymorphisms of SNAP-25 have been associated with attention deficit/hyperactivity disorder (Galvez et al., 2014). Thus, we hypothesized that the expression of SNAP-25 is associated with sensory function or locomotor function in rats with SCI. In the present study, we aimed to investigate whether SNAP-25 was associated with SCI and determine its role in SCI.

Materials and Methods

Animals

A total of 240 adult female Sprague-Dawley rats aged 12 weeks and weighing 200–220 g were purchased from the

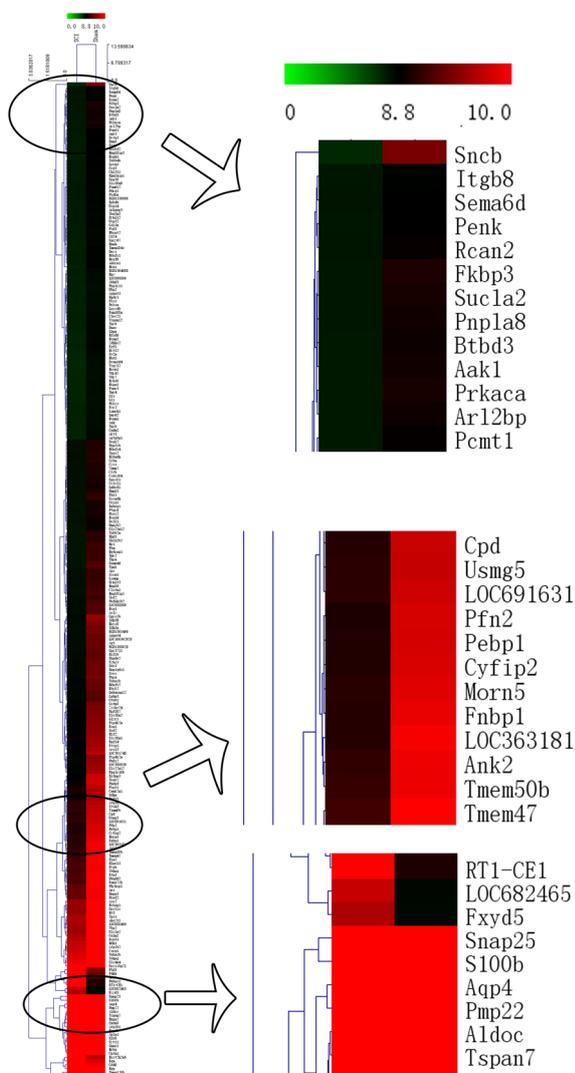


Figure 1 Results of gene array in the spinal cord of rats with spinal cord contusion injury.

The cluster heat map shows mRNA expression between 1.5- and 2.5-fold changes in gene array data ($P < 0.05$). Thirteen genes were upregulated, whereas 249 genes were downregulated in the SCI group compared with the sham group. SCI: Spinal cord injury.

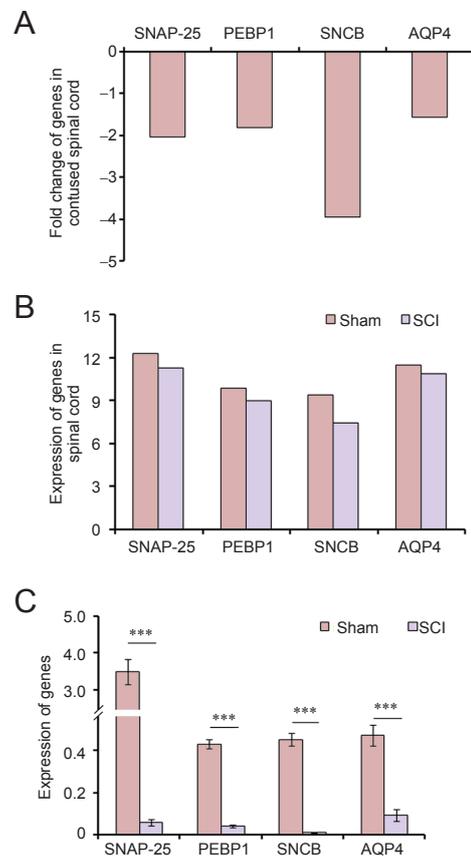


Figure 3 Significant genes in the spinal cord of rats with spinal cord contusion injury.

(A) The fold change shows that SNCB was highest among SNAP-25, PEBP1, SNCB and AQP4 genes. (B) The expression of SNAP-25, PRBP1, SNCB and AQP4. SNAP-25 had the highest expression in both groups based on the gene array. Data are shown as the logarithmic function of 2. (C) Quantitative real time-polymerase chain reaction was used to verify the accuracy of the gene array. The levels of SNAP-25, PRBP1, SNCB and AQP4 were significantly decreased in the SCI group when compared with the sham group, and SNAP-25 was most highly expressed in the two groups. Thus, SNAP-25 was selected for further research in this study. Data are relative to beta-actin. Data are presented as the mean \pm SEM and were analyzed using Student's *t*-test. *** $P < 0.001$. SCI: Spinal cord injury; SNAP-25: synaptosomal-associated protein 25 kDa; PEBP1: phosphatidylethanolamine binding protein 1; SNCB: beta-synuclein; AQP4: aquaporin 4.

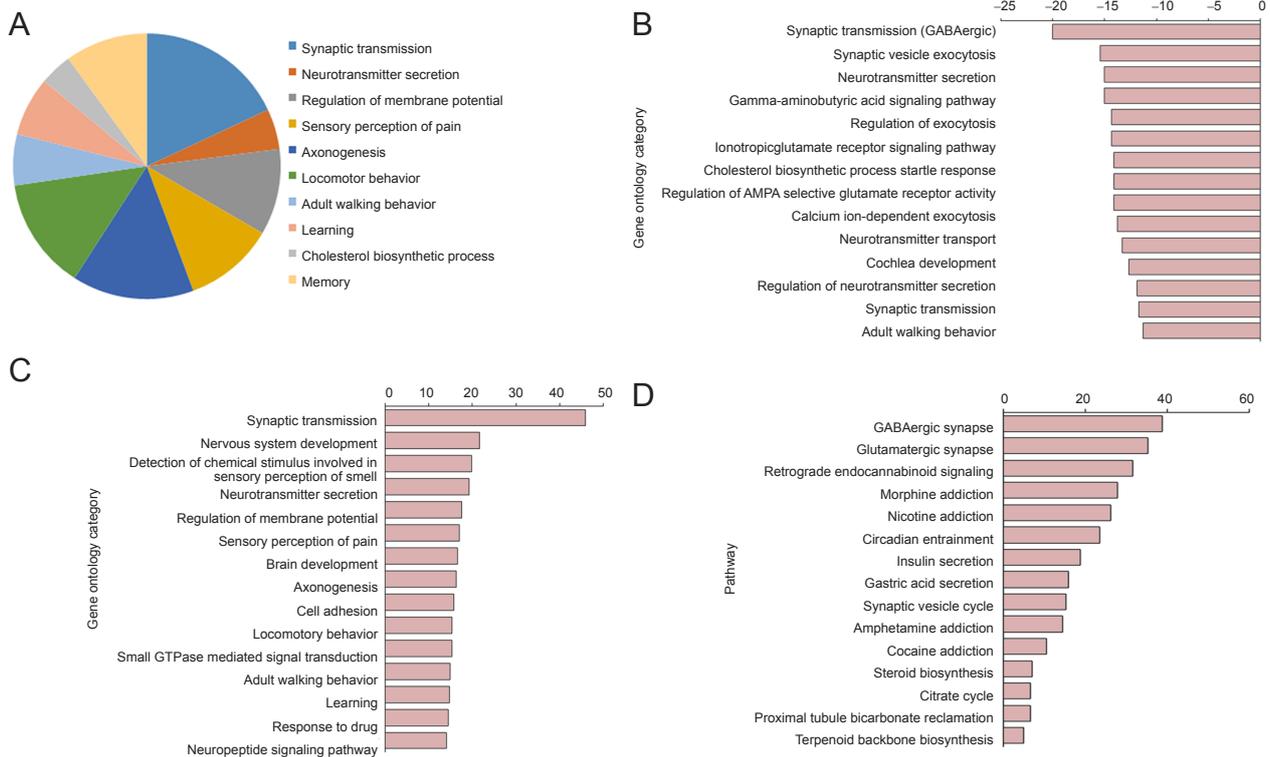


Figure 2 Analysis of downregulated genes in the spinal cord of rats with spinal cord contusion injury.

(A) We focused on the downregulated genes, which comprised the majority of the significantly different genes identified with the cluster heat map. The top 10 classifications were identified based on the number of differential genes in different biological processes. (B) The top 15 classifications according to the enrichment of significantly downregulated genes ($P < 0.001$) in biological processes. (C) The top 15 biological process classifications according to the negative of log function of P values ($P < 0.001$) of significantly downregulated genes. (D) The top 15 classifications based on the pathway analysis of significantly downregulated genes. GO: Gene ontology.

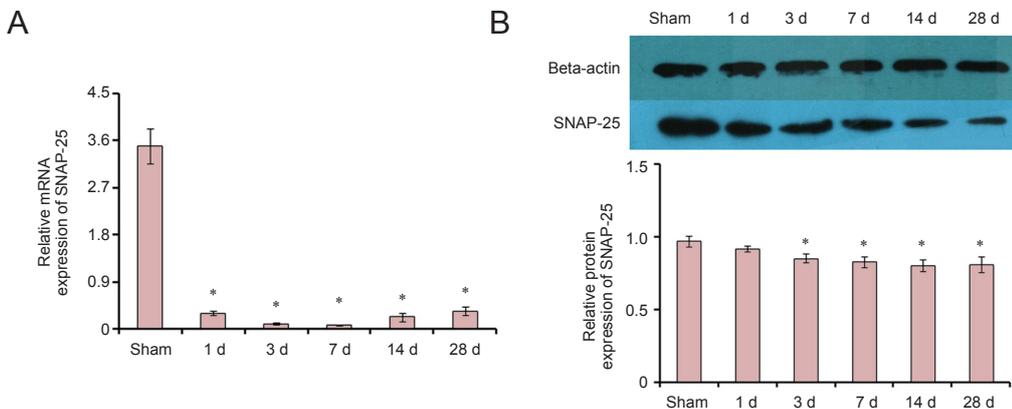


Figure 4 SNAP-25 expression in rats with spinal cord injury.

(A) SNAP-25 expression in spinal cord tissue of rats detected by quantitative real time-polymerase chain reaction. Data are relative to beta-actin. Data are presented as the mean \pm SEM and were analyzed using one-way analysis of variance with Dunnett T3. (B) Western blot assay was used to detect SNAP-25 protein expression in the spinal cord. Data represent the optical density ratio to beta-actin. Data are presented as the mean \pm SEM and were analyzed using one-way analysis of variance with the least significant difference. * $P < 0.05$, vs. sham group. SNAP-25: Synaptosomal-associated protein 25 kDa; d: day(s).

Dashuo of Laboratory Animal Co., Ltd., Chengdu, China (SYXK (Chuan) 2014-189). Rats were randomly divided into a sham group ($n = 120$) and a SCI group ($n = 120$), and the survival periods for subgroups were 1, 3, 7, 14 and 28 days post injury, with 24 rats in each group.

Rats were maintained in strict conditions at 22–25°C, 30–40% humidity and 12/12-hour light/dark cycles. All

animal care and experiments were performed in accordance with the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals (Publication No. 85-23, revised 1986). This research was approved by the Institutional Animal Care and Use Committee, Chengdu Medical College, China (approval number: 201401120411).

Table 1 Gene sequences

Gene	Forward (5'-3')	Reverse (5'-3')	Renaturation temperature (°C)
β-Actin	GAA GAT CAA GAT CAT TGC TCC T	TAC TCC TGC TTG CTG ATC CA	52
SNAP-25	AGG ACT TTG GTT ATG TTG GAT	GAT TTA AGC TTG TTA CAG G	50
PEBP1	ATG CTC CCA GCA GGA AGG A	GAC AGT GCC ACT GCT AAT	53
SNCB	TCC TCT ACG TCG GAA GCA AG	CCA CTT CCT CTG GCT TCA G	55
AQP4	GAC ATT TGT TTG CAA TCA AT	AAC CCA ATA TAT CCA GTG GTT	52

SNAP-25: Synaptosomal-associated protein 25 kDa; PEBP1: phosphatidylethanolamine binding protein 1; SNCB: beta-synuclein; AQP4: aquaporin 4.

SCI induction

Surgical procedures were performed in a standard method as previously described (Zhang et al., 2015). In brief, after anesthesia with 3.6% chloral hydrate (1 mL/100 g; Kelong, Chengdu, Sichuan Province, China), the position of tenth thoracic vertebra was identified and the skin was sterilized. A laminectomy was performed, exposing the T₁₁ spinal cord. A weight-drop device (Chengdu Taimeng, Chengdu, Sichuan Province, China) was used to injure the spinal cord, with a drop of 30 mm. The sham group received only a laminectomy without injury. Rats received benzylpenicillin (80,000 U/kg/d, intramuscularly) and normal saline (5 mL, intramuscularly) after surgery. Urinary bladders were manually expressed until autonomous micturition recovery.

Gene array and gene ontology (GO) category analysis

Rats in the sham group and 3-day group ($n = 3$) were anesthetized with 3.6% chloral hydrate, and tissue from the SCI site was taken and used for gene array. Purified RNA was performed using Affymetrix GeneChip Gene 2.0 Array (Genminix Informatics Ltd., Co., Shanghai, China) according to the manufacturer's protocol.

GO category analysis was used to classify hierarchical categories and predict the most effective differential genes based on the biological process. GO category analysis was used to analyze the data in the gene array. A *t*-test was used to detect the difference between the two groups. False discovery rate was used to determine whether the difference was significant by random chance, with $P < 0.001$ and false discovery rate < 0.05 (Chen et al., 2010).

Quantitative real time-polymerase chain reaction (qRT-PCR)

qRT-PCR (Applied Biosystems, Waltham, MA, USA) was used to verify the differential expression of genes that were detected by the Affymetrix GeneChip. The procedures were conducted as described previously (Chen et al., 2005). The gene sequences are shown in **Table 1**. The expression levels of genes were shown as an increase in cycle threshold relative to the expression of β-actin. The relative gene expression in the sample was calculated as $2^{-\Delta\Delta CT}$ (Meng et al., 2011).

To detect the change of SNAP-25 levels in the spinal cord, the center of the contused spinal cord was obtained from the sham and SCI groups (1, 3, 7, 14 and 28 days, $n = 6$ per group) after rats were anesthetized with 3.6% chloral hydrate and perfused with normal saline (Kelun, Chengdu, China). RNA was extracted from spinal cord with Trizol reagent

(Invitrogen, Waltham, MA, USA), cDNA was obtained with RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA), and the amplified primers of the gene were list in **Table 1**. All of the procedures were conducted according to the manufacturer's protocol.

The target gene was detected at 95°C for 2 minutes and amplified at 95°C for 15 seconds, followed by the appropriate renaturation temperature (**Table 1**) for 20 seconds and 60°C for 40 seconds. All steps were performed for 45 cycles. Statistical analyses were performed as described for the GO analysis.

Western blot assay

Spinal cord tissues were harvested as described previously. Tissues were homogenized in protein extraction reagent (Beyotime Biotechnology, Nantong, China). In detail, proteins were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride membrane (Millipore, Billerica, MA, USA) in transfer buffer. Membranes were blocked by 5% skim milk and incubated with monoclonal rabbit anti-SNAP-25 (1:200; Boster, Wuhan, Hebei Province, China) or mouse anti-beta-actin (1:4,000; ProteinTech, Chicago, IL, USA) overnight at 4°C. Horseradish peroxidase-conjugated goat anti-rabbit or goat anti-mouse IgG (1:10,000; ProteinTech) antibodies were applied for 2 hours at 37°C after membranes were washed by Tris-buffered saline containing 0.5% Tween-20. Membranes were detected by Enhanced Chemiluminescence (Beyotime Biotechnology) and exposure to film in the dark. The optical density intensity of each band was measured using Quantity One software (BioRad, Waltham, MA, USA). Results were shown as the optical density ratio to beta-actin.

Test of pain thresholds and locomotor function

To evaluate the change of sensory function in SCI rats, the latency of the hind limbs' withdrawal response to thermal stimuli (Chengdu Taimeng) was assessed. The rats in the sham group and 28-day group ($n = 15$) were subjected to thermal stimuli before surgery, and 1, 7, 14, 21 and 28 days after injury. Each rat was measured three times with a 3-minute interval between tests.

To detect the change of motor function after SCI, rats were tested with the Basso, Beattie, Bresnahan Locomotor Rating Scale (BBB) (Basso et al., 1995). The rats in the sham group and 28-day group ($n = 15$) were measured at 1, 7, 14, 21 and 28 days after surgery. Rats were allowed to move in an open field for 5 minutes. A lower BBB score indicates worse locomotor function.

Thermal stimuli tests and BBB assessments were performed by three experts and independent observers. All of them were blinded to the treatments of each group.

Immunohistochemistry

Immunohistochemistry was used to detect changes in morphology and SNAP-25 expression in the posterior and anterior horns of spinal cord after SCI. The primary antibody was mouse anti-SNAP-25 (1:100, monoclonal, Boster). Rats in the SCI and sham groups ($n = 3$ per group) were anesthetized with 3.6% chloral hydrate and perfused with 4% paraformaldehyde. Briefly, spinal cord tissues were fixed in paraffin. Paraffin sections were cut transversely at 4 μm . After dewaxing and hydration, high-pressure antigen retrieval was performed. The sections were rinsed in 0.05 M phosphate-buffered saline and endogenous enzymes were inactivated by 3% H_2O_2 . Before adding the primary antibody, 5% goat serum with 0.3% Triton X-100 was used. Sections were incubated overnight at 4°C with the primary antibody followed by horseradish peroxidase-conjugated goat anti-mouse IgG (1:200; ZSGB-BIO, Beijing, China). Images of the epicenter of the injured spinal cord were photographed using an Olympus BX-DSU microscope (Olympus, Tokyo, Japan). The positive staining was quantified using ImageJ (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

All of the data were analyzed by SPSS 17.0 (SPSS, Chicago, IL, USA). Values are presented as the mean \pm standard error of the mean. Data were analyzed using one-way analysis of variance with the least significant difference or Student's *t*-test. Values of $P < 0.05$ were considered statistically significant.

Results

Gene expression changes in the spinal cord of rat models of SCI

A gene array was used to detect changes of gene expression in the sham and SCI groups. Based on a 1.5–2.5-fold change in the gene array data, displayed as a heat-map, 13 genes were upregulated and 249 genes were downregulated in the SCI group compared with the sham group (**Figure 1**). Because a majority of the significant genes were downregulated on the cluster heat map, we chose the downregulated genes for further examination. The top 10 implicated biological processes were identified based on the number of different genes in each biological process (**Figure 2A**). In addition, the top 15 significantly downregulated genes are shown in **Figure 2B**, and the negative of the log function of *P*-values of the significantly downregulated genes in the biological processes are shown in **Figure 2C**.

To further clarify the affected processes in the spinal cord, we analyzed the pathways of significantly downregulated genes. The top 15 identified pathways are involved in: GABAergic synapse, glutamatergic synapse, retrograde endocannabinoid signaling, morphine addiction, nicotine addiction, circadian entrainment, insulin secretion, gastric acid secretion, synaptic vesicle cycle, amphetamine addiction, cocaine

addiction, steroid biosynthesis, citrate cycle, proximal tubule bicarbonate reclamation and terpenoid backbone biosynthesis (**Figure 2D**).

According to the analyses of biological processes, the downregulated genes were involved in synaptic transmission, neurotransmitter secretion, sensory perception of pain, axonogenesis, locomotor behavior and adult walking behavior.

SNAP-25 chosen for further analysis

From the results of the differential genes combined with the biological process analysis, we pursued SNAP-25, phosphatidylethanolamine binding protein 1 (PEBP1), beta-synuclein (SNCB) and aquaporin 4 (AQP4) genes. The fold change of SNCB was highest among them (**Figure 3A**). SNAP-25 exhibited the highest expression in the SCI and sham groups based on gene array (**Figure 3B**). Finally, qRT-PCR was used to verify the accuracy of the gene array analysis. The levels of SNAP-25, PEBP1, SNCB and AQP4 were significantly decreased in the SCI group when compared with the sham group (**Figure 3C**), and SNAP-25 was most highly expressed in the two groups. In summary, we selected SNAP-25 for further research in this study.

SNAP-25 expression in rats with SCI

To detect the change of SNAP-25 expression in rats with SCI, we performed qRT-PCR and western blot assay in spinal cord tissue. SNAP-25 mRNA expression was significantly decreased at 1, 3, 7, 14 and 28 days (**Figure 4A**). Furthermore, SNAP-25 protein expression was significantly reduced at 3, 7, 14 and 28 days (**Figure 4B**). These results showed that SNAP-25 expression might be associated with SCI.

SNAP-25 associated with sensory function in rats with SCI

We hypothesized that SNAP-25 was involved in the sensory function of SCI rats, and therefore measured pain sensitivity. The latency of the hind limb response to the mechanical stimulus was reduced at 7, 14, 21 and 28 days when compared with the sham group (**Figure 5**). To investigate the localization of SNAP-25 in the spinal cord, we performed immunohistochemistry. Dense immunoreactivity was found on axons of neurons and glial cells in the dorsal horn of the spinal cord in the sham and SCI groups (**Figure 6A–G**). The density of SNAP-25 immunoreactivity at 1, 3, 7, 14 and 28 days was reduced in the SCI group compared with the sham group (**Figure 6H**). The results suggest that reduced levels of SNAP-25 are associated with sensory function in rats with SCI.

SNAP-25 associated with locomotor function in rats with SCI

We examined the locomotor behavior of rats in both the SCI and sham groups. Locomotor function decreased immediately after SCI, but gradually increased over time. The BBB scores were significantly lower in the SCI group than in the sham group at each time point (**Figure 7**). Similarly, the BBB scores of SCI rats at 7 and 14 days were significantly higher than at 1 and 7 days, respectively.

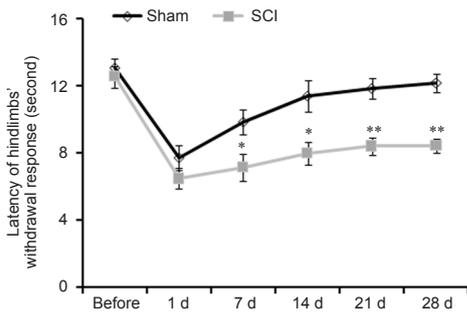


Figure 5 Changes of pain sensitivity in rats with SCI. Data are presented as mean ± SEM and were analyzed using Student's *t*-test. **P* < 0.05, ***P* < 0.01, vs. sham group. SCI: Spinal cord injury; d: day(s).

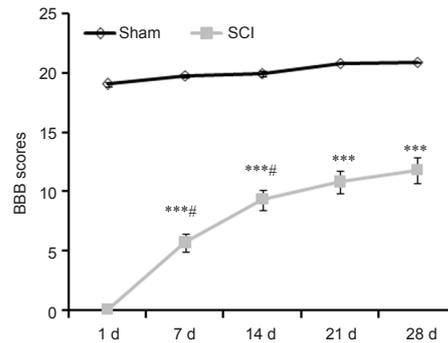


Figure 7 Change of locomotor function in rats with SCI. Lower BBB scores indicate worse locomotor function. Data are presented as the mean ± SEM. The difference was analyzed between sham and SCI groups using one-way analysis of variance with the least significant difference, and the difference was compared at each time point using Student's *t*-test. ****P* < 0.001, vs. sham group; #*P* < 0.05, vs. the previous time point. SCI: Spinal cord injury; BBB: Basso, Beattie, Bresnahan Locomotor Rating Scale.

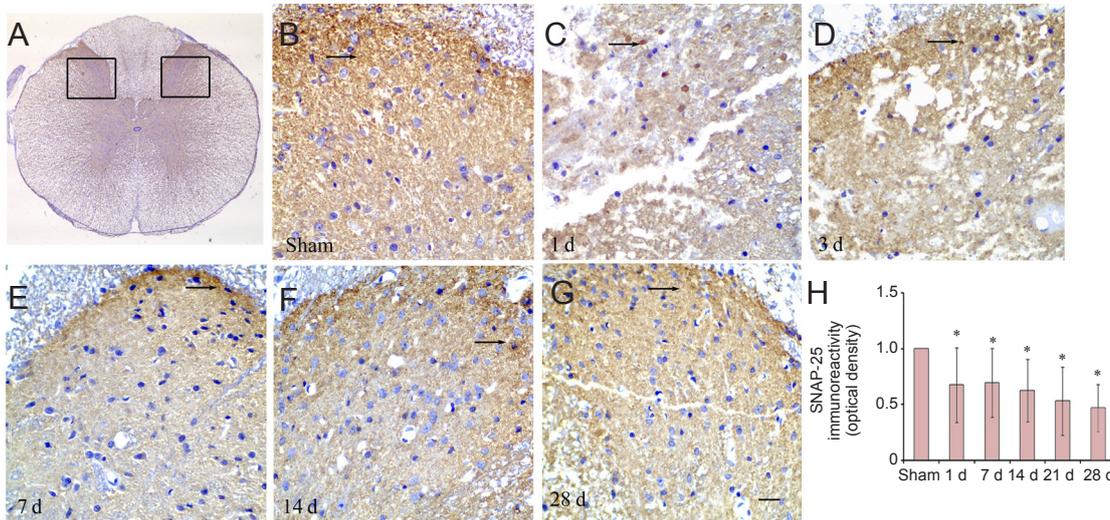


Figure 6 Distribution of SNAP-25 in the posterior horn of spinal cord. Immunohistochemistry was performed to investigate the location of SNAP-25 in the spinal cord of rat. (A) The region of SNAP-25-immunoreactivity located in the posterior horn of spinal cord. The squares correspond to the location of images in B–G. (B–G) A large amount of immunoreactivity was found on axons of neurons and glial cells in the dorsal horn of the spinal cord in both the sham and spinal cord injury groups. Scale bar: 100 μm. Black arrows indicate SNAP-25 immunoreactivity. (H) Quantitative results of the density of SNAP-25 immunoreactivity. Data are presented as the mean ± SEM and were analyzed using one-way analysis of variance with the least significant difference. **P* < 0.05, vs. sham group. SNAP-25: Synaptosomal-associated protein 25 kDa; d: day(s).

Interestingly, the immunoreactivity of SNAP-25 was located in the anterior horn of the spinal cord, in which the majority of immunoreactivity was localized in the axon or cytoplasm of the neurons (Figure 8A–G). The number of SNAP-25-immunoreactive cells significantly decreased at 1, 3, 14 and 28 days (Figure 8H). Thus, we presumed that the decrease of SNAP-25 expression was associated with the locomotor function after SCI.

Discussion

In this study, we speculated that SNAP-25 might be associated with sensory function and locomotor function in an SCI rat model. Gene array and GO analysis were used to identify significantly different genes in SCI rats. Results of qRT-PCR

and western blot assay showed that SNAP-25 expression decreased after SCI. Furthermore, immunohistochemistry and behavioral tests demonstrated that SNAP-25 was located in the posterior and anterior horns of the spinal cord, and was associated with sensory function and locomotor function in SCI rats. Therefore, SNAP-25 may be beneficial to neural regeneration following SCI, and might be a potential target gene for the treatment of SCI.

An association of SNAP-25 levels with clinical symptoms suggest that SNAP-25 plays a role in disease. We hypothesized that SNAP-25 was associated with SCI. In this study, SNAP-25 gene and protein expression levels were significantly lower in the SCI group than in the sham group, which indicates that SNAP-25 has a direct or indirect relationship with the SCI

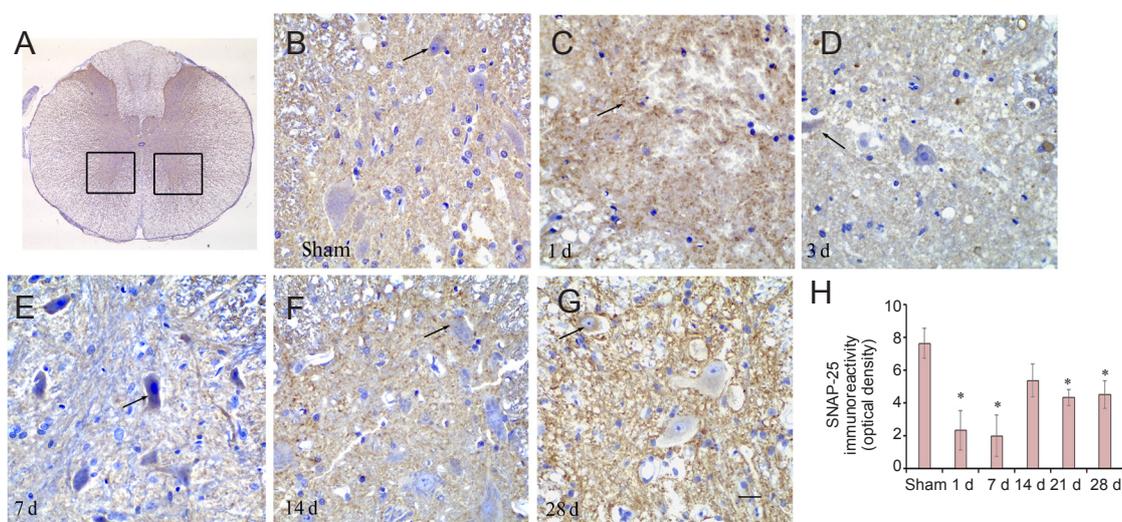


Figure 8 Distribution and role of SNAP-25 in the anterior horn of spinal cord.

(A) SNAP-25 immunoreactivity was located in the anterior horn of spinal cord. The squares correspond to the location of images in B–G. (B–G) Representative images of SNAP-25 immunohistochemistry in the anterior horn of the spinal cord, in which the majority of immunoreactivity was localized in neuronal axons and cytoplasm. Scale bar: 100 μ m. Black arrows indicate SNAP-25 immunoreactivity in axons or cytoplasm. (H) Quantitative results of SNAP-25 immunoreactivity. Data are presented as the mean \pm SEM and were analyzed using one-way analysis of variance with the least significant difference. * $P < 0.05$, vs. sham group. SNAP-25: Synaptosomal-associated protein 25 kDa; d: day(s).

contusion. Our results are consistent with previous studies, which show that SNAP-25 expression is decreased in children affected by autism spectrum disorders and in patients with diverticular disease (Barrenschee et al., 2015; Braida et al., 2015). However, SNAP-25 protein expression was significantly higher in cerebrospinal fluid when measured using affinity purification and mass spectrometry in Alzheimer's disease (Brinkmalm et al., 2014). Fibromyalgia syndrome patients have an increased frequency of a SNAP-25 gene polymorphism compared with matched healthy women, which is associated with the behavioral, personality and psychological disorders in fibromyalgia syndrome patients (Balkarli et al., 2014). Our results differ with the findings from these two reports, which occurred because SNAP-25 might play different roles in different diseases or in different regions of the peripheral and central nervous systems. For example, SNAP-25 was downregulated in the hippocampus of Alzheimer's disease (Kamat et al., 2016).

Neuropathic pain is a characteristic feature of SCI (Tan and Waxman, 2015). Recently, a study has shown that SNAP-25 is associated with the function of protein kinase C gamma that regulates neuropathic pain in rat models of chronic constriction injury-induced neuropathic pain (Zou et al., 2012). Additionally, Liu et al. (2013) has found that SNAP-25 is correlated with the relief of nociceptive responses in chronic constriction injury rats through botulinum toxin serotype A treatment. In this study, SNAP-25 immunoreactivity was found in the posterior horn of the spinal cord, and the density of SNAP-25 immunoreactivity was significantly reduced after SCI. Thus, we presume that SNAP-25 is associated with neuropathic pain. Interestingly, we detected the latency of hind limb withdrawal response to temperature stimulation and found that the latency was reduced after SCI, which is similar to previous reports (Houeland et al., 2007; Wang et al., 2014) suggesting

that SNAP-25 has an important role in sensory function.

Dysfunction after SCI has an influence on the overall well-being of patients. In this study, SNAP-25 immunoreactivity was expressed in the neuronal cytoplasm of the anterior horn of the spinal cord, and behavioral function was impaired in SCI rats. Thus, we speculate that SNAP-25 is involved in the locomotor function of SCI rats. SNAP-25 is widely distributed in the nervous system and plays an essential role in different structures, such as in the hippocampus and enteric nerve cells (Barrenschee et al., 2015; Yang et al., 2015). Islamov et al. (2015) have shown that SNAP-25 is synthesized in motor nerve endings and that the level of SNAP-25 mRNA affects neurotransmitter exocytosis, suggesting that SNAP-25 plays an important role in the motor neurons of the spinal cord. Reduced levels of SNAP-25 mRNA and protein in the brain of a mutant mouse model termed coloboma show that upregulated SNAP-25 in the spinal motor neurons preserves vital neuromuscular function (Adler et al., 2001). *In vivo*, acute downregulation of SNAP-25 in the CA1 hippocampal region affects spine number, which demonstrates that SNAP-25 may contribute to disease pathology through an effect on postsynaptic function and plasticity (Fossati et al., 2015). Cultures of rat superior cervical ganglion neurons reveal that SNAP-25 is also involved in neurite extension and arborization (Lawrence et al., 2014).

We suggest that SNAP-25 may influence the recovery of locomotor function after SCI *via* axon plasticity and neural regeneration. Together, our findings support that a decreased level of SNAP-25 plays an important role in SCI rats, which might be associated with sensory function and locomotor function. SNAP-25 provides a new direction for the role of SNAP-25 in SCI.

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Author contributions: ZQZ, XZ (Xiao Zhang) and THW designed the experiments. ZQZ wrote the main manuscript text. YCW and XZ (Xi Zeng) prepared all figures. ZQZ and YX conducted the western blot experiments. ZQZ, YX and XZ (Xi Zeng) established animal models. XMW, YCW and XH conducted the behavioral tests. QJX conducted qRT-PCR. ZQZ and XZ (Xi Zeng) conducted the immunohistochemistry. All authors approved the final version of the paper.

Conflicts of interest: None declared.

Research ethics: The study protocol was approved by the Institutional Animal Care and Use Committee, Chengdu Medical College, China (Approval number: 201401120411). The experiment followed the National Institutes of Health Guide for the Care and Use of Laboratory animals (NIH Publications No. 8023, revised 1978), and "Consensus Author Guidelines on Animal Ethics and Welfare" produced by the International Association for Veterinary Editors (IAVE). The article was prepared in accordance with the "Animal Research: Reporting of In Vivo Experiments Guidelines" (ARRIVE Guidelines).

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