Regional cerebral metabolic levels and turnover in awake rats after acute or chronic spinal cord injury

Liang Wu1,2,3,4 | Zhanfeng Niu1 | Xulei Hu1,3,4 | Huili Liu2,5 | Shuang Li2 | Lei Chen2 | Danhao Zheng2,5 | Zhuang Liu2,5 | Taotao Liu6 | Fuqiang Xu2,5 | Anne Manyande7 | Jie Wang2,5,8 | Hechun Xia1,9

1Department of Neurosurgery, General Hospital of Ningxia Medical University, Yinchuan, P.R. China
2Key Laboratory of Magnetic Resonance in Biological Systems, State Key Laboratory of Magnetic Resonance and Atomic and Molecular Physics, Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, Innovation Academy for Precision Measurement Science and Technology, Wuhan, P.R. China
3Ningxia Key Laboratory of Cerebrocranial Diseases, Yinchuan, P.R. China
4School of Clinical Medicine, Ningxia Medical University, Yinchuan, P.R. China
5University of Chinese Academy of Sciences, Beijing, P.R. China
6Department of Anesthesiology, Peking University Third Hospital, Beijing, P.R. China
7School of Human and Social Sciences, University of West London, London, UK
8Hebei Provincial Key Laboratory of Basic Medicine for Diabetes, 2nd Hospital of Shijiazhuang, Shijiazhuang, P.R. China
9Ningxia Human Stem Cell Research Institute, General Hospital of Ningxia Medical University, Yinchuan, P.R. China

Hechun Xia, Department of Neurosurgery, General Hospital of Ningxia Medical University, Yinchuan, Ningxia, 750004, P.R. China.
Email: xhechun@nyfy.com.cn

Jie Wang, Key Laboratory of Magnetic Resonance in Biological Systems, State Key Laboratory of Magnetic Resonance and Atomic and Molecular Physics, Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, Innovation Academy for Precision Measurement Science and Technology, Wuhan, Hubei, 430071, P.R. China.
Email: jie.wang@wipm.ac.cn

Abstract

Spinal cord injury (SCI) is a common cause of disability, which often leads to sensorimotor cortex dysfunction above the spinal injury site. However, the cerebral regional effects on metabolic information after SCI have been little studied. Here, adult Sprague-Dawley rats were divided into acute and chronic treatment groups and sham groups with day-matched periods. The Basso, Beattie, and Bresnahan scores method were utilized to evaluate the changes in behaviors during the recovery of the animals, and the metabolic information was measured with the 1H-observed/13C-edited NMR method. Total metabolic concentrations in every region were almost similar in both treated groups. However, the metabolic kinetics in most regions in the acute group were significantly altered (P < .05), particularly in the cortical area, thalamus and medulla (P < .01). After long-term recovery, some metabolic kinetics were recovered, especially in the temporal cortex, occipital cortex, and medulla. The metabolic kinetic changes revealed the alteration of metabolism and neurotransmission in

Abbreviations: Asp, aspartic acid; BBB, Basso, Beattie, and Bresnahan; CE, cerebellum; Cr, creatine; FC, frontal cortex; GABA, γ-aminobutyric acid; Glx, glutamine; Glu, glutamate; HP, hippocampus; HYP, hypothalamus; MED, medulla; MID, midbrain; NAA, N-acetyl-aspartic acid; NMR, nuclear magnetic resonance; OC, occipital cortex; PC, parietal cortex; POCE, 1H observed/13C-edited; PQN, probabilistic quotient normalization; SCI, spinal cord injury; STR, striatum; TC, temporal cortex; THA, thalamus.

Liang Wu and Zhanfeng Niu are co-authors and contributed equally to this article.
1 | INTRODUCTION

Spinal cord injury (SCI) is a common neurological injury that is associated with functional deficits and is also a major cause of disability. In traumatic SCI, the primary insult damages cells and initiates a complex secondary injury cascade, which cyclically produces the death of neurons and glial cells, ischemia and inflammation.1 Secondary injury, which occurs hours to months after the initial primary traumatic insult, contributes to metabolic stress and progressive tissue damage and serves as a prime target for therapeutic intervention.2 Although numerous neuroprotective, neural regenerative, and rehabilitation exercise therapies have been translated from preclinical studies into clinical trials, to date, there are no efficient or reliable clinical treatments available for SCI patients. One potential reason holding back improvements in SCI therapy lies in current strategies which focus on local changes at the spinal injury site and neglect the intimate interconnection with the brain.3

In recent years, there has been increasing evidence that SCI leads to alterations in brain structure, function and metabolism, by direct effects of nerve damage, secondary mechanisms, and also by longer term injury consequences such as paralysis and neuropathic pain.4 Several studies have shown that SCI resulted in central nervous system injury and structural reorganization of the spine and brain.5,6 Cortical functional reorganization in the sensorimotor areas has been demonstrated in the later stages of SCI.7,8 In addition, the deafferentation and loss of sensorimotor function after SCI not only directly impacts the sensorimotor system, but also influences other regions, such as the insular, cerebellar, medial prefrontal, anterior cingulate, and temporal cortices, which are crucial for processing emotional information and modulating attentional states.9 SCI also induces multiple disturbances in the metabolic network, including oxidative stress, glycolysis, amino acid, and lipid metabolism.10,11 Rapid release of excessive glutamate and other neurotransmitters that may directly contribute to cellular damage has been observed following SCI.12 However, previous studies have mainly focused on the SCI site or local area, hence the cerebral regional effects on metabolic information after SCI have been little studied, such as metabolite concentrations and metabolic kinetics of neurotransmitters and some other energetically related neurochemicals.

After SCI, mitochondria dysfunction occurred in the brain during the acute phase, followed by inflammatory response and ER stress aroused at subacute phase.13 While normal brain function requires a stable energy supply, disturbances in brain energy metabolism have been associated with neurological dysfunctions and cognitive impairment.14 Glucose is considered as the main substrate for neuronal energy metabolism in the mammalian brain.15 It has also been estimated that the cycling between glutamine (Gln) and glutamate (Glu) accounts for more than 80% of cerebral glucose consumption.10 The tight coupling between the Glu–Gln cycle and brain energetics is largely tied to the nearly 1:1 stoichiometry between glucose oxidation and the rate of astrocytic Glu uptake.17 Thus, it is valuable to investigate the imbalance of brain energy metabolism in neurons and astrocytes and explore the pathogenesis of neurological disorders after SCI.18

1H observed/13C-edited (POCE) nuclear magnetic resonance (NMR) technique is a promising approach for investigating the metabolic kinetics in astrocytes, specific neurons, and their interactions.19 Metabolic information between neuronal and astrocytic interaction can be investigated by the POCE method combined with the infusion of 13C-labeled glucose/acetate.20-22 We hypothesized that SCI could produce lasting deficits in brain metabolism. Thus, the purpose of the present study was to evaluate the effect of SCI on regional metabolic concentrations and rates of turnover of glutamate, glutamine, and GABA (γ-aminobutyric acid) and other metabolites in the rat brain. The changes in metabolic information could reveal the influence on different brain regions, which could present evidence for the alternation of regional cerebral glucose oxidation and cerebral function after SCI. Furthermore, this study could provide the theoretical basis for clinical therapy after SCI, such as mitochondrial transplantation.

2 | METHODS

2.1 | Animals

The experimental protocols were approved by the animal care and use committee in Wuhan Institute of Physics and Mathematics, the Chinese Academy of Sciences. In order to
investigate the changes in metabolic information among different brain regions after spinal cord injury, the severe SCI model was used in the current study. However, this surgery can cause great trauma to animals, with many postoperative complications such as urinary retention and high mortality. Due to their different physiological structures, there is a much higher mortality rate in male rats than in female rats, even when the bladder is massaged two or three times per day. Therefore, to save the number of animals, female rats are often used in this kind of studies.23-25

In the current study, 52 female adult Sprague-Dawley rats (n = 12 for each group in the NMR study, and n = 4 for the histology study) were ordered from VITAL RIVER (Beijing, China) and kept in SPF (Specific pathogen Free) animal residence (Wuhan, China). Rats were housed in plastic cages (three animals per cage) in a climate-controlled room with 12 hours of light-dark illumination cycle at 25 ± 1°C and relative 50 ± 10% humidity. During the experiment, all rats were allowed free access to laboratory standard food (Product No: 190011304, WQJX Biotech, Wuhan, China) and kept in SPF (Specific pathogen Free) animal residence. Due to failure related to animal surgery (n = 3) and tail vein catheterization (n = 4), seven of the 48 rats were not included in the data analysis.

2.2 Animal experiment

At first, all animals were randomly divided into four equal groups: the acute SCI treatment group (3 days after injury), chronic SCI treatment group (28 days after injury) and the sham controls with day-matched periods (without SCI). Each subject was given a unique identification number, and the information of the experimental group was blinded to the operators, which could potentially influence outcomes of the experimental groups.

For animal surgery, a rat was anesthetized with 1% pentobarbital (i.p., 5 mg/100 g). A sagittal incision was made at the lower dorsal part of the thoracic segment to expose the T7-T9 vertebral plate and spinous processes. The T8 vertebral plate was cut and removed under a surgical microscope. The SCI was induced by contusion (200 kdyne) to the exposed segment, resulting in a severe contusion injury. Then the muscle layers and skin layers were sutured together after contusion. Animals in the control group only suffered sagittal incision and laminectomy for spinal cord exposure, but without contusion by the impactor. After the operation, all animals were carefully monitored for their mental status, such as eating, drinking, urination, as well as edema and ulcers. A Water Gel pack and food pellets were provided at the bottom of the cage for up to 72 hours after SCI. Penicillin was continuously injected at 100 000 units/time/day up to 7 days. The bladder was massaged two to three times per day until recovery of spontaneous urinary function. At the end, two rats died during the operation procedures and one died from paralytic intestinal obstruction.

2.3 Basso, Beattie, and Bresnahan locomotor scale

In order to assess the motor function, all animals in the chronic SCI group and its related control group were placed in an uninterrupted open field and allowed unrestricted movement. Rats were allowed to move freely and scored for their ability to use their hindlimbs. A 21-point BBB locomotion scale was used based on the movement of joints, placement of paws and coordination of forepaw and hind limbs.26 The BBB scores were determined 0, 1, 2, 3, and 4 weeks post-SCI to assess recovery of locomotion in the chronic SCI treatment group.

2.4 Perfusion and histology of the spinal cord

Rats from sham (Acute) (n = 2) and Acute SCI (n = 2) groups were anesthetized with 1% pentobarbital (i.p., 6 mg/100 g), and transcardially perfused with 0.9% saline (~300 mL, room temperature), followed by buffered 4% formaldehyde solution. The spinal cord of the eighth thoracic segment was taken, fixed in 4% formaldehyde for 24 hours, then embedded with alcohol gradient dehydration and paraffin. The horizontal and transverse sections of the spinal cord were cut and stained with HE (Hematoxylin eosin staining) and Nissl (Nissl’s staining) for histopathology examination under a microscope (Leica, Wetzlar, Germany).

2.5 Infusion techniques

The metabolic kinetics were assessed with the $^{13}$C enrichment into different carbon positions of metabolites after the infusion of [1-$^{13}$C] glucose. For this method, the higher enrichment of [1-$^{13}$C] glucose in the blood could yield greater sensitivity for detection. Therefore, the rats were fasted overnight (15-18 hours) to reduce the endogenous unlabeled glucose level before the experimental day.

On the experimental day, rats were initially anesthetized with 4.0%-5.0% isoflurane mixed with air, and 1.5%-2.5% isoflurane to maintain the anesthesia state. The adequate level of anesthesia was verified by a lack of withdrawal response to a foot pinch. Then, one lateral tail vein was catheterized with PE50 tubing (Instech, PA, USA) for the infusion of [1-$^{13}$C] glucose, and the tube was immobilized to the tail.
with adhesive paper tape. Then those animals were recovered for about 15 minutes until they showed free movement and normal grooming. The infusion line was connected to a swivel (Instech, PA, USA) and suspended from the center of the cage to avoid entanglement of the line during the rat movement. The other end of the swivel was connected to the infusion pump (Fusion100, Chemyx, TX, USA) with PE50 tubing. After everything was set up, the animal was allowed to recover for another 15 minutes. Then, [1-13C] glucose was infused through the lateral tail vein following the former infusion protocol\(^\text{22}\) and the infusion ceased after 20 minutes. During the whole procedure, the rat had freedom of movement in the cage. All animals were sacrificed by the head-focused microwave irradiation method (1 kW, Tangshan Nanosource Microwave Thermal Instrument Manufacturing Co. Ltd., Heibei, PR China). Then a blood sample (~1 mL) was withdrawn and the brain was manually dissected into 11 different regions as described previously:\(^\text{22}\) cerebellum (CE), medulla (MED), midbrain (MID), thalamus (THA), hypothalamus (HYP), hippocampus (HP), striatum (STR), frontal cortex (FC), occipital cortex (OC), parietal cortex (PC), and temporal cortex (TC). The tissue was weighed, frozen in liquid nitrogen, and stored at −80°C until further processing. Four rats failed due to very low [1-13C] glucose enrichment (<10%), caused by the failure of tail vein catheterization. Thus, the cerebral data from these animals were ignored. Therefore, there were 41 rats that successfully completed the study, including 20 rats from the two separate control groups (10 by 10) and 21 rats from the two SCI groups (nine for acute SCI and 12 for Chronic SCI).

### 2.6 Sample collection and preparation

The preparation of brain tissue extracts was conducted using the same methanol-ethanol extraction method which was described in our previous work.\(^\text{27}\) Briefly, HCl/methanol (80 μL, 0.1 M) was added to the brain samples, and the tissues were initially homogenized with TissueLyser (TissueLyser II, QIAGEN, German) for 90 seconds at a frequency of 20 Hz. Then, 400 μL of ethanol (60%, vol/vol) was added to the mixture and the mixture was homogenized under the same conditions. The homogenate was centrifuged at 14 000 \(g\) for 15 minutes and the supernatant was collected. The entire extraction procedure was repeated twice with 1200 μL of 60% ethanol. All the supernatants were collected and lyophilized with the centrifugal drying apparatus (Thermo Scientific TissueLyser II, Germany) after removing the organic solvent (ethanol and methanol) in the vacuum under normal temperature. The lyophilized products were re-dissolved in phosphate buffer (600 μL of \(D_2O\) with 0.2 M Na\(_2\)HPO\(_4\)/NaH\(_2\)PO\(_4\), pH 7.2). The solution was mixed evenly with a high-speed vortex and centrifuged at 14 000 \(g\) for 15 minutes, and the supernatant (500 μL) was transferred to a 5 mm NMR tube for \(^1\)H-NMR analysis.

### 2.7 Acquisition of NMR spectra

All NMR spectra were acquired in a random order at 298 K using a BrukerAvance III 600 MHz NMR vertical bore spectrometer (Bruker Biospin, Germany). The samples were detected with POCE (Proton observed carbon editing, \(^1\)H-[\(13\)C]-NMR) pulse sequence which has been widely used for \(^13\)C enrichment in different positions of metabolites after infusion of \(^13\)C-labeled chemical tracer.\(^\text{21}\) Briefly, this method consists of two spin-echo measurements, one without a broad-banded inversion pulse applied at the \(^13\)C frequency (total metabolites concentrations, \(^12\)C+\(^13\)C), and the other one with the inversion pulse (the difference of the proton signals which connected with \(^12\)C and \(^13\)C in the metabolites, \(^12\)C−\(^13\)C). Thus, the subtraction between these two yields only \(^13\)C-labeled metabolites of the spectra. The following acquisition parameters were used: number of scans—64; repetition time—20 seconds; sweep width—20 ppm; acquisition data—64 K; echo time—8 ms.

### 2.8 NMR spectra processing

All FID signals of \(^1\)H-NMR spectra were converted and the phase and baseline correction were manually performed in the commercial software Topspin 2.1 (Bruker Biospin GmbH, Rheinstetten, Germany). Then the spectra were automatically processed with a home-made software NMRSpec\(^\text{28}\) in MATLAB (Freely available from the author upon request: jie.wang@wipm.ac.cn).

### 2.9 Relative concentration calculation

At first, the phase and baseline corrected POCE spectra were loaded into NMRSpec. Then the peak alignment, integrations of peaks and chemical-related peaks were automatically completed. The extract ratio for a sample was an unpredicted value, and it was hardly the same as the others. Therefore, it was better to complete the normalization before further analysis. All peak areas and spectra data were normalized with the conventional probabilistic quotient normalization (PQN) method,\(^\text{29}\) which has been widely used in metabolomics researches.\(^\text{30,31}\) For the relative concentration calculation, the average chemical-related peak area in the Sham (Acute) group was set as reference “1,” then the relative concentrations of this metabolite in every sample was calculated from the quotient between the same location of the NMR spectrum and that averaged peak area. Then the average concentration and standard error of various metabolites...
in different experimental groups were calculated according to the method described above.

2.10 Metabolic enrichment calculation

The $^{13}$C-related NMR spectrum was obtained by subtracting the two spin-echo measurements in the POCE spectrum ($2 \times ^{13}$C), and the $^{13}$C fractional enrichment was calculated from the ratio between this $^{13}$C-related NMR spectrum and the non-edited ($^{12}$C+$^{13}$C) spectrum. Thus, this value is not related to the tissue weights and the extraction ratios, and the original peak integrations in the POCE spectrum used for the analysis.

2.11 Data analysis

In this study, all rats were randomly assigned to the experimental procedures including housing and feeding. Single-factor analyses of variance were performed to determine the difference in the level of metabolites, $^{13}$C fractional enrichment in different positions of metabolites, and BBB scores. Differences of the amino acid levels and $^{13}$C fractional enrichments were identified with Student $t$ test with adjustment of $P$ value for Bonferroni correction. All results were presented by mean ± SEM.

3 RESULTS

3.1 Basso, Beattie, and Bresnahan locomotor scale

The BBB score was used to evaluate and compare the motor function and recovery of the animals in the chronic SCI treatment group with the chronic sham group. The BBB score for the chronic SCI group (n = 12) showed improvement with a mean initial score of 6.00 ± 0.84 in the first week which increased to a mean score of 15.08 ± 1.71 by the fourth week (Figure 1). Based on the results of the comparison, the animal gradually recovered during the first 3 weeks, and reached the optimum level around the third week. In addition, some of the rats had recovered well by the end of the fourth week (n = 4).

3.2 SCI leads to neurons death and glial cells activation

In the sham group, the gray and white matter structures of the spinal cord are clear, the cells are evenly distributed and orderly arranged, neurons and glial cells are clearly visible, and no cavities and necrotic tissues are observable (Figure 2).

3.3 POCE NMR spectrum of brain extracts

In order to evaluate the total concentrations of metabolites and metabolic kinetics in different brain regions, the POCE NMR pulse sequence was employed to investigate the metabolic compositions of the brain extracts in the current study. Here a typical series of POCE NMR spectra for four different groups are illustrated in Figure 3. The relative concentrations of the metabolites were obtained from the PQN normalized non-edited spectrum (upper four spectra); and the total concentrations of $^{13}$C-labeled metabolites were calculated by subtracting the two series of spectra in POCE data which are shown in the lower four spectra.

3.4 Metabolites concentration in different brain regions after SCI

To explore the changes of metabolites in different brain regions, which might be related to neurobehavioral
**FIGURE 2**  HE and Nissl staining of the spinal cord (horizontal and transverse sections) after SCI. The structures of spinal cord are clear, neurons (Long arrows) and glial cells (Short arrows) orderly arranged in sham group. Neurons disappear and glial cells migrate into the lesion site, transforming into foam cells (Arrowheads) in SCI group. scale bar = 500/300/100 μm, applicable to all corresponding sections.

**FIGURE 3**  NMR spectra for total metabolites (12C+13C, upper) and 13C-related metabolites (2*13C, lower) from the POCE (1H observed/13C edited) NMR spectra for the frontal cortex. Subscript: proton signal connected with the 13C position in the metabolites; Asp, aspartate; Cre, creatine; GABA, γ-aminobutyric acid; Gln, glutamine; Glu, glutamate; Glx, glutamine + glutamate; NAA, N-acetylaspartate.
abnormalities after SCI, the relative concentrations of metabolites in four different groups (Acute SCI vs. Sham (Acute); Chronic SCI vs. Sham (Chronic)) were compared among 11 brain regions. After comparison, the concentrations of most metabolites did not change in these two pairs. For example, several metabolite concentrations (glutamate, GABA, and aspartic acid (Asp)) are illustrated in Figure 4. For these metabolites, there were only a few changes among 22 pair comparisons in each metabolite (two group pair × 11 brain regions). Glutamate was significantly decreased only in FC for the chronic SCI group (Figure 4A, P = .002). However, there were opposite changes observed for GABA in the cortex and deep brain areas, and GABA was increased in HYP (P = .008) and TC (P = .035, Figure 4B). Furthermore, there was an increase in Asp at regions of MED and HP (P = .004 and .008, Figure 4C). However, changes in metabolite concentration after SCI were similar in both acute and chronic groups. In order to pursue the influence of SCI on brain function, it was valuable to investigate the changes of the 13C enrichment in various metabolic positions for different groups during [1-13C]-glucose infusion.

3.5 | Metabolic 13C enrichments in different brain regions

With the infusion of [1-13C]-glucose, different positions of metabolites were labeled via the tricarboxylic acid (TCA) cycles in GABAergic and glutamatergic neurons and astroglia cells. For the first TCA cycles in neurons, Glu4 (glutamatergic neuron), and GABA2 (GABAergic neuron) were labeled with 13C probe, and Glu4 was labeled in astroglia cells. Then the other carbon positions in metabolites were gradually labeled with further TCA cycles.

For the acute SCI model, 13C enrichments in different positions of metabolites among most brain regions were decreased, especially for the cortex (FC, OC, PC, and TC), MID, MED, and HYP (Figures 5, 6 and S1). After chronic SCI treatment, the metabolic kinetics in the cortex was recovered, especially for OC and TC (Figures 5B and S1). However, the 13C enrichments in some regions were decreased, such as THA and CE (Figures 6A and S1). In order to show the tendency of changes, 13C enrichment in different positions of metabolites for the cortex (FC and TC) and sub-cortex (THA and HP) are illustrated in the main text (Figures 5 and 6), respectively.

Most enrichment of 13C-labeled amino acids from [1-13C] glucose in the prefrontal cortex of different groups exhibited significant changes. The 13C enrichments in Glu4 (P = .037), Asp3 (P = .028), GABA3 (P = .048) and Glx3 (P = .046) in the acute SCI group were found to be significantly lower than in the acute sham group. In addition, Glu4 (P = .011), Asp3 (P = .001), Glu3 (P = .023), GABA3 (P = .016), Glx2 (P = .023), and Ala (P = .001) in the chronic SCI group was also found to be significantly different from the chronic sham group (Figure 5A). For the temporal cortex, there were only significant differences observed in the acute SCI group, such as Glu4 (P = .029), GABA3 (P = .035), Asp3 (P = .004), Glu3 (P = .016), Glx3 (P = .022), and Glx2 (P = .026), and only Glu4 (P = .043) in the temporal cortex dramatically changed in the chronic SCI group (Figure 5B).

Compared with the acute SCI treatment, THA had more fractional 13C enrichment in different types of metabolites in the chronic SCI group, such as Glu4 (P = .005), GABA4 (P = .005), Asp3 (P = .001), GABA3 (P = .033), Glx3 (P = .018), Glx2 (P = .011), and Ala3 (P = .01). However, only Glu4 (P = .047) and Glx4 (P = .047) were decreased in the acute SCI group (Figure 6A). The enrichments of Glu4 and Glx4 were significantly decreased in the hippocampus in both groups (Figure 6B). Furthermore, some metabolic kinetics changed dramatically after long-term recovery, especially in the temporal cortex, occipital cortex and medulla (Figure S1).

4 | DISCUSSION

Spinal cord injury is defined as damage to the spinal cord which temporarily or permanently causes changes in its structure and function, and the structural dysfunction can induce the changes of metabolic activity in the central nervous system. The cerebral regional effects on the metabolic information are also known to be closely associated with changes in the cerebral structure and function.

4.1 | Metabolite concentrations and SCI

Alternations of metabolic information have been used as biological markers for more widespread physiological changes in the brain and SCI site. Recent studies have described metabolic changes in cortical activation during sensory and/or motor tasks in cervical myelopathy and patients with SCI.3,32 It has been shown that the levels of NAA (N-acetyl-aspartic acid), Cr (Creatine), Ins, and Glu were increased in the thalamus/ striatum of rats after SCI.33 However, another study reported that NAA and GABA levels were reduced in the thalamus of SCI patients with neuropathic pain compared to those without pain and healthy controls.34 The current study also focused on examining the metabolic changes in different brain regions after SCI. However, there were few changes in metabolic concentrations in most brain regions which is not consistent with former findings. Compared with previous work, the current 1H-NMR method used has much higher signal to noise value than the in vivo MRS approach.33,34 Furthermore, the
FIGURE 4  The relative concentrations of metabolites in 11 different brain regions for four different groups (Sham (Acute): 12; Acute SCI: 11; Sham (Chronic): 10; and Chronic SCI: 12). A, Glutamate, (B), GABA, (C), Aspartic acid; Values represent mean ± SEM; *P < .05, **P < .01
tissue volume detected by the in vivo MRS method is always standard cuboid or square shapes, which is not consistent with the real shape of the brain region. In this study, the cerebral regional tissues were dissected and the metabolites extracted and measured using the ¹H-NMR method which is more accurate compared to the in vivo method.

4.2 Metabolic kinetics and SCI

In general, the metabolic kinetics in most brain regions were significantly decreased in both acute and chronic groups. Thus, SCI was observed to exert significant effects on the TCA cycle, which mainly occurs in mitochondria, and generates ATP and metabolites for survival and growth. Mitochondria dysfunction in the brain always occurred during acute SCI, followed by the inflammatory response and ER stress aroused in the subacute phase, which plays a key role in the development of secondary pathophysiology after contusion SCI. The results highlight an acute and chronic deficit in mitochondrial bioenergetics associated with SCI that may lead to a novel approach for neural restoration after SCI.

With different methods targeting mitochondria dysfunction, multiple groups have reported that this yields neuroprotection, tissue sparing, and functional recovery. Mitochondrial transplantation is emerging as a potential therapeutic to maintain mitochondrial function after injury, consequently improving chronic functional outcome. Although this therapy is relatively new, mitochondrial transplantation is effective in promoting recovery after ischemic injury to cardiac tissue. Many pharmacological agents that have proven beneficial for the treatment of SCI in vivo to some extent affect mitochondria or mitochondrial function. For example, the antibiotic minocycline was found to have neuroprotective effects and induced behavioral and cellular recovery after SCI in rats. NACA treatment significantly maintained acute mitochondrial bioenergetics and normalized GSH levels following SCI, and the prolonged delivery resulted in significant tissue sparing and improved recovery of hind limb function.

**FIGURE 5** The ¹³C fractional enrichments in different kinds of metabolites from [¹-¹³C] glucose in the frontal cortex (A) and temporal cortex (B) for four different groups. Values represent mean ± SEM. *P < .05, **P < .01; C₂-C₄: proton signals connected with the related ¹³C positions (2-4) in the metabolites.
Regional effects and SCI

The metabolic information, required to determine metabolic fluxes, commonly vary with cerebral regions as shown in the human, rat, and mouse brains. Both the cerebral cortex and the deep brain regions have their own variation characteristics after SCI. Comparisons of metabolic enrichment in the cerebral cortex, thalamus, and hippocampus revealed substantial and highly significant regional variations (Figures 5 and 6).

In previous studies, the cerebral cortex was selected as a whole region in order to compare regional changes in metabolite concentrations. However, the regional cerebral effects on metabolic information in the brain cortex after SCI have been little studied. The present study revealed changes in different brain cortices and showed distinct variations of metabolic kinetics in the cortex. The frontal cortex showed that the 13C enrichment in neurotransmitters were markedly decreased in both chronic and acute groups. However, the impact of SCI on the TC (Figure 5B) and OC (Figure S1B) were mainly significantly decreased in the acute group, and the alterations were mostly recovered after long-term recovery. A greater understanding of how sensory-motor function reorganize, both spontaneously after injury and in response to therapeutic interventions, is necessary in order to develop repair strategies that maximize function and are readily translatable to clinical practice. It has recently been shown that, in humans, physiotherapy can improve neurocognitive deficits associated with SCI. From the finding in the present study, it could be proposed that metabolic changes in cortical regions (sensorimotor) reasonably overlap with the ones positively affected by physiotherapy in humans. Thus, the present study offers an animal-based neurophysiological explanation of a behavioral effect observed in humans. Furthermore, this study also provides the neurophysiology-based understanding for designing brain-machine interfaces that could restore the lost motor function for improving rehabilitation.

The reorganization observed at the cortical level could also occur at the subcortical level. Nonetheless, subcortical reorganization could in principle occur either in the thalamus or brainstem. The thalamus plays a central role in modulating the selection, execution, modification of motor...
programs, nociception, and almost all sensory modalities. The changes after SCI lead to the reorganization of the thalamus. The present study revealed changes in the thalamus that showed a higher TCA cycle flux compared with the hippocampus, which suggests distinct kinetics in the rat. The $^{13}$C enrichment amino acids in the thalamus mainly decreased in the chronic group, while only Glu$_4$ and Gln$_4$ were significantly decreased in the acute group, which might indicate that SCI in rats could produce lasting deficits in thalamus metabolism.

### 4.4 Neuronal types and SCI

The impact of SCI on excitatory transmitters (Glu) was greater than on inhibitory neurotransmitters (GABA) in most brain regions, especially for the acute SCI group (Cerebral alterations -Figures 5, 6 and S1: 9 regions for Glu$_4$ and 2 regions for GABA$_4$). Glutamate and glutamine are relatively abundant amino acids in the brain that are critical for neuronal function, and they were involved in the regulation of brain energy metabolism. A dysfunction in the homeostasis, recycling, and metabolism of glutamate also participates in the course of many chronic neurodegenerative diseases. Alteration of this cyclic nature of Glu and Gln has been shown to play an important role in the regulation of various neurological disorders, including epilepsy, multiple sclerosis, traumatic brain injury, schizophrenia, and brain tumors.

Neuropathic pain after SCI was also reported to be always associated with altered thalamic anatomy, biochemistry, and activity, which may result in disturbed thalamocortical circuits. The glutamatergic metabolism, glial proliferation, glial hypertrophy, or activation might be factors contributing to intense neuropathic pain after SCI. There is no doubt that unbalanced metabolism of neurotransmitters may be involved in the process of neuropathic pain. Specifically, it has been shown that neuropathic pain after SCI is associated with changes in thalamic neurons, which subsequently may make these neurons hyperexcitable, and as such, may act as a pain generator or amplifier. Hyperexcitability also plays a role in the genesis of multi-sensory symptoms after SCI, this might either be a common phenomenon across different sensory cortices, or one might postulate hyperexcitability within a structure with sensory input to the thalamus. Thus, the current study speculates that the glutamatergic activity could be related to neuropathic pain caused by SCI, which could provide an avenue for the clinical therapy of SCI.

### 5 CONCLUSION

In this report, the metabolite levels were almost similar in every cerebral region during the different stages of SCI, but the metabolic kinetics ($^{13}$C fractional enrichment in different carbon positions of metabolites) were significantly lower in most regions, especially the frontal cortex, parietal cortex, hippocampus, thalamus, and hypothalamus in both acute and chronic SCI groups. After long-term recovery, some metabolic kinetics were recovered, especially in the temporal cortex, occipital cortex, and medulla. Furthermore, the impact of SCI on excitatory transmitters (Glu) was greater than on inhibitory neurotransmitters (GABA) in most brain regions, especially for the acute SCI group. The changes in metabolic kinetics revealed that the alteration in metabolism and neurotransmission in different brain regions could present evidence for the alternation of brain glucose oxidation after SCI. Therefore, SCI significantly influenced the cerebral function, especially for acute intervention.

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### CONFLICT OF INTEREST

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

### AUTHOR CONTRIBUTIONS

JW, FX, and HX contributed to experimental design. LW, ZN, LC, DZ, ZL, XH, AM, SL, HL, and TL contributed to animal experiment data acquisition and data analysis. LW, JW, and HX contributed to data analysis, result interpretation, and writing. All authors have read, revised, and approved the final manuscript.

### DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this article.

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**SUPPORTING INFORMATION**

Additional Supporting Information may be found online in the Supporting Information section.

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