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

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Running head: Cerebral metabolic level after spinal cord injury

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Nonstandard abbreviations list: SCI: Spinal cord injury; BBB: Basso, Beatti and Bresnahan; Gln: Glutamine; Glu: Glutamate; Asp: Aspartic acid; NAA: N-acetyl-aspartic acid, Cr: Creatine; GABA: γ-aminobutyric acid; POCE: ^{1}H observed/^{13}C-edited; NMR: Nuclear magnetic resonance; CE: Cerebellum; MED: Medulla; MID: Midbrain; THA: Thalamus; HYP: Hypothalamus; HP: Hippocampus; STR: Striatum; FC: Frontal cortex; OC: Occipital cortex; PC: Parietal cortex; TC: Temporal cortex; PQN: Probabilistic quotient normalization.

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 BBB (Basso, Beatti and Bresnahan) scores method was utilized to evaluate the changes in behaviors during the recovery of the animals, and the metabolic information was measured with the ^{1}H-observed/^{13}C-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 ($p<0.05$), particularly in the cortical area, thalamus and hippocampus ($p<0.01$). 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 different brain regions after SCI, which present evidence for the alternation of brain glucose oxidation. Therefore, this shows the significant influence of SCI on cerebral function and neuroscience research. This study also provides the theoretical basis for clinical therapy after SCI, such as mitochondrial transplantation.

***Keywords*:** Spinal cord injury; Neurotransmitters; Metabolic kinetics; Brain regions; NMR;

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, ischaemia 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 metabolite, 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 have 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 (16). 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).

^{1}H observed/^{13}C-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 ^{13}C-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.

Methods

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 of metabolic information among different brain regions after spinal cord injury, the severe spinal cord injury (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 messaged 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 h of light-dark illumination cycle at 25±1 ºC 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 water. 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.

120 *Animal experiment*

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

126 For animal surgery, a rat was anesthetized with 1% pentobarbital (*i.p.*, 5mg/100g). A sagittal
127 incision was made at the lower dorsal part of the thoracic segment to expose the T7-T9 vertebral plate
128 and spinous processes. The T8 vertebral plate was cut and removed under a surgical microscope to
129 expose the intact dura. The animal was transferred to beneath the IH impactor which was equipped
130 with a 4 mm tip, and the incision site was centered. The SCI was induced by contusion (200 kdyne) to
131 the exposed segment, resulting in a severe contusion injury. Then the muscle layers and skin layers
132 were sutured together after contusion. Animals in the control group only suffered sagittal incision and
133 laminectomy for spinal cord exposure, but without contusion by the impactor. After the operation, all
134 animals were carefully monitored for their mental status, such as eating, drinking, urination, as well
135 as edema and ulcers. A Water Gel pack and food pellets were provided at the bottom of the cage for
136 up to 72 h after SCI. Penicillin was continuously injected at 100000 units/time/day up to 7 days. The
137 bladder was massaged 2-3 times per day until recovery of spontaneous urinary function. At the end,
138 two rats died during the operation procedures and one died from paralytic intestinal obstruction.

139

140 *Basso, Beattie, and Bresnahan (BBB) Locomotor Scale*

141 In order to assess the motor function, all animals in the chronic SCI group and its related control
142 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.

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.*, 6mg/100g), 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).

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 (15-18h) 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 min. Then, [1-^{13}C] glucose was infused through the lateral tail vein following a former infusion protocol (22) and the infusion ceased after 20 minutes. During the whole procedure, the rat had freedom of movement in the cage (22). All animals were sacrificed by the head-focused microwave irradiation method (1kW, Tangshan Nanosource Microwave Thermal Instrument Manufacturing Co. Ltd., Heibei, P.R. China). Then a blood sample (~1 mL) was withdrawn and the brain was manually dissected into 11 different regions as described previously (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℃ until further processing. Four rats failed due to very low [1-^{13}C] 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 (9 for acute SCI and 12 for Chronic SCI).

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 (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 90s at a frequency of 20 Hz. Then, 400 μL ethanol (60%,

190 *vol*/*vol*) was added to the mixture and the mixture was homogenized again under the same conditions.
191 The homogenate was centrifuged at 14000 *g* for 15 min and the supernatant was collected. The entire
192 extraction procedure was repeated twice with 1200 μL 60% ethanol. All the supernatants were
193 collected and lyophilized with the centrifugal drying apparatus (Thermo Scientific 2010, Germany)
194 after removing the organic solvent (ethanol and methanol) in the vacuum under normal temperature.
195 The lyophilized products were re-dissolved in phosphate buffer (600μL D_2O with 0.2 M
196 Na_2HPO_4/NaH_2PO_4, pH=7.2). The solution was mixed evenly with a high-speed vortex and
197 centrifuged at 14000 *g* for 15 min, and the supernatant (500 μL) was transferred to a 5 mm NMR tube
198 for ^{1}H-NMR analysis.
199
200 *Acquisition of NMR spectra*
201 All NMR spectra were acquired in a random order at 298 K using a BrukerAvance III 600 MHz
202 NMR vertical bore spectrometer (BrukerBiospin, Germany). The samples were detected with POCE
203 (Proton observed carbon editing, ^{1}H -[^{13}C]-NMR) pulse sequence which has been widely used for ^{13}C
204 enrichment in different positions of metabolites after infusion of ^{13}C labeled chemical tracer (21).
205 Briefly, this method consists of two spin-echo measurements, one without a broad-banded inversion
206 pulse applied at the ^{13}C frequency (total metabolites concentrations, ^{12}C+^{13}C), and the other one with the
207 inversion pulse (the difference of the proton signals which connected with ^{12}C and ^{13}C in the metabolites,
208 ^{12}C-^{13}C). Thus, the subtraction between these two yields only ^{13}C-labeled metabolites of the spectra. The
209 following acquisition parameters were used: number of scans - 64; repetition time – 20 s; sweep width
210 - 20 ppm; acquisition data - 64 K; echo time-8 ms.
211

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 was automatically processed with a home-made software NMRSpec (28) in MATLAB (Freely available from the author upon request: jie.wang@wipm.ac.cn).

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 (29), which has been widely used in metabolomics research (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.

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.

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.

Results

Basso, Beattie and Bresnahan (BBB) 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 (Fig. 1). Based on the results of the comparison, the animal gradually recovered during the first three weeks, and reached the optimum level around the 3rd week. In addition, some of the rats had recovered well by the end of the 4th week (n=4).

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 (Fig. 2). In the acute SCI group, the structure of the injury site is disordered, and the boundary between the gray matter and white matter of the spinal cord is unclear.

Cavities and necrotic tissues can be seen, neurons disappeared, and a large number of activated glial cells migrated into the lesion site, transforming into foam cells under phagocytosis (Fig. 2). Thus, the animal model for the SCI treatment was successfully constructed.

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 Fig. 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.

Metabolites concentration in different brain regions after SCI

To explore the changes of metabolites in different brain regions, which might be related to neurobehavioral 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 Fig. 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 (Fig. 4A, p=0.002). However, there were opposite changes observed for GABA in the cortex and deep brain areas, and GABA was increased in HYP (p=0.008) and TC (p=0.035, Fig.4B). Furthermore, there was an increase in Asp at regions of MED and HP (p=0.004 and

278 0.008, Fig. 4C). However, changes in metabolite concentration after SCI were similar in both acute
279 and chronic groups. In order to pursue the influence of SCI on brain function, it was valuable to
280 investigate the changes of the ^{13}C enrichment in various metabolic positions for different groups during
281 [1-^{13}C]-glucose infusion.

282 *Metabolic ^{13}C enrichments in different brain regions*

283 With the infusion of [1-^{13}C]-glucose, different positions of metabolites were labelled *via* the
284 tricarboxylic acid (TCA) cycles in GABAergic and glutamatergic neurons and astroglia cells. For the
285 first TCA cycles in neurons, Glu_4 (glutamatergic neuron) and $GABA_2$ (GABAergic neuron) were
286 labeled with ^{13}C probe, and Gln_4 was labeled in astroglia cells. Then the other carbon positions in
287 metabolites were gradually labelled with further TCA cycles.

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

295 Most enrichment of ^{13}C labeled amino acids from [1-^{13}C] glucose in the prefrontal cortex of
296 different groups exhibited significant changes. The ^{13}C enrichments in Glu_4 (p=0.037), Asp_3 (p=0.028),
297 $GABA_3$ (p=0.048) and Glx_3 (p=0.046) in the acute SCI group were found to be significantly lower
298 than in the acute sham group. In addition, Glu_4 (p=0.011), Asp_3 (p=0.001), Glu_3 (p=0.023), $GABA_3$
299 (p=0.016), Glx_2 (p=0.023) and Ala (p=0.001) in the chronic SCI group was also found to be

300 significantly different from the chronic sham group (Fig. 5A). For the temporal cortex, there were only
301 significant differences observed in the acute SCI group, such as Glu_4 (p=0.029), $GABA_3$ (p=0.035),
302 Asp_3 (p=0.004), Glu_3 (p=0.016), Glx_3 (p=0.022) and Glx_2 (p=0.026), and only Glu_4 (p=0.043) in the
303 temporal cortex dramatically changed in the chronic SCI group (Fig. 5B).
304 Compared with the acute SCI treatment, THA had more fractional ^{13}C enrichment in different
305 types of metabolites in the chronic SCI group, such as Glu_4 (p=0.005), $GABA_4$ (p=0.005), Asp_3
306 (p=0.001), $GABA_3$ (p=0.033), Glx_3 (p=0.018), Glx_2 (p=0.011) and Ala_3 (p=0.01). However, only Glu_4
307 (p=0.047) and Gln_4 (p=0.047) were decreased in the acute SCI group (Fig. 6A). The enrichments of
308 Glu_4 and Gln_4 were significantly decreased in the hippocampus in both groups (Fig. 6B).
309
310 **Discussion**
311 SCI is defined as damage to the spinal cord which temporarily or permanently causes changes in
312 its structure and function, and the structural dysfunction can induce the changes of metabolic activity
313 in the central nervous system. The cerebral regional effects on the metabolic information are also
314 known to be closely associated with changes in the cerebral structure and function.
315 *Metabolite concentrations and SCI*
316 Alternations of metabolic information have been used as biological markers for more widespread
317 physiological changes in the brain and SCI site. Recent studies have described metabolic changes in
318 cortical activation during sensory and/or motor tasks in cervical myelopathy and patients with SCI (7,
319 32). It has been shown that the levels of NAA (N-acetyl-aspartic acid), Cr (Creatine), Ins and Glu were
320 increased in the thalamus/striatum of rats after SCI (33). However, another study reported that NAA
321 and GABA levels were reduced in the thalamus of SCI patients with neuropathic pain compared to

322 those without pain and healthy controls (34). The current study also focused on examining the
323 metabolite changes in different brain regions after SCI. However, there were few changes in metabolic
324 concentrations in most brain regions which is not consistent with former findings. Compared with
325 previous work, the current ^{1}H-NMR method used has much higher signal to noise value than the *in*
326 *vivo* MRS approach (33, 34). Furthermore, the tissue volume detected by the *in vivo* MRS method is
327 always standard cuboid or square shapes, which is not consistent with the real shape of the brain region.
328 In this study, the cerebral regional tissues were dissected and the metabolites extracted and measured
329 using the ^{1}H-NMR method which is more accurate compared to the *in vivo* method.
330
331 *Metabolic kinetics and SCI*
332 In general, the metabolic kinetics in most brain regions were significantly decreased in both acute
333 and chronic groups. Thus, SCI was observed to exert significant effects on the TCA cycle, which
334 mainly occurs in mitochondria, and generates ATP and metabolites for survival and growth (35).
335 Mitochondria dysfunction in the brain always occurred during acute SCI, followed by the
336 inflammatory response and ER stress aroused in the subacute phase (36), which plays a key role in the
337 development of secondary pathophysiology after contusion SCI (37). The results highlight an acute
338 and chronic deficit in mitochondrial bioenergetics associated with SCI that may lead to a novel
339 approach for neural restoration after SCI.
340 With different methods targeting mitochondria dysfunction, multiple groups have reported that
341 this yields neuroprotection, tissue sparing, and functional recovery (38, 39). Mitochondrial
342 transplantation is emerging as a potential therapeutic to maintain mitochondrial function after injury,
343 consequently improving chronic functional outcome (40). Although this therapy is relatively new,

mitochondrial transplantation is effective in promoting recovery after ischemic injury to cardiac tissue (41). Many pharmacological agents that have proven beneficial for the treatment of SCI *in vivo* to some extent affect mitochondria or mitochondrial function (39). For example, the antibiotic minocycline was found to have neuroprotective effects and induced behavioral and cellular recovery after SCI in rats (42). 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 hindlimb function (43).

Regional effects and SCI

The metabolic information, required to determine metabolic fluxes, commonly vary with cerebral regions as shown in the human (44), rat (45) and mouse (46) brains. Both the cerebral cortex and 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 (Fig. 5 and 6).

In previous studies, the cerebral cortex was selected as a whole region in order to compare regional changes in metabolite concentrations (45). 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 ^{13}C enrichment in neurotransmitters were markedly decreased in both chronic and acute groups. However, the impact of SCI on the TC (Fig. 5B) and OC (Fig. S1B) were mainly significantly decreased in the acute group (Fig. 5), and the alterations were mostly recovered after long-term recovery. A greater understanding of how sensory-motor function reorganize,

366 both spontaneously after injury and in response to therapeutic interventions, is necessary in order to
367 develop repair strategies that maximize function and are readily translatable to clinical practice. It has
368 recently been shown that, in humans, physiotherapy can improve neurocognitive deficits associated
369 with SCI (47). From the finding in the present study, it could be proposed that metabolic changes in
370 cortical regions (sensorimotor) reasonably overlap with the ones positively affected by physiotherapy
371 in humans. Thus, the present study offers an animal-based neurophysiological explanation of a
372 behavioral effect observed in humans. Furthermore, this study also provides the neurophysiology-
373 based understanding for designing brain–machine interfaces that could restore the lost motor function
374 for improving rehabilitation (48).

375 The reorganization observed at the cortical level could also occur at the subcortical level (49).
376 Nonetheless, subcortical reorganization could in principle occur either in the thalamus (50) or
377 brainstem (51). The thalamus plays a central role in modulating the selection, execution, modification
378 of motor programs, nociception and almost all sensory modalities (52). The changes after SCI lead to
379 the reorganization of the thalamus (53). The present study revealed changes in the thalamus that
380 showed a higher TCA cycle flux compared with the hippocampus, which suggests distinct kinetics in
381 the rat. The ^{13}C enrichment amino acids in the thalamus mainly decreased in the chronic group, while
382 only Glu_4 and Gln_4 were significantly decreased in the acute group, which might indicate that SCI in
383 rats could produce lasting deficits in thalamus metabolism.

384

385 *Neuronal types and SCI*

386 The impact of SCI on excitatory transmitters (Glu) was greater than on inhibitory
387 neurotransmitters (GABA) in most brain regions, especially for the acute SCI group (Cerebral

alterations -Fig. 5, 6 and S1: 9 regions for Glu_4 and 2 regions for $GABA_2$). Glutamate and glutamine are relatively abundant amino acids in the brain that are critical for neuronal function (54), and they were involved in the regulation of brain energy metabolism (18). A dysfunction in the homeostasis, recycling, and metabolism of glutamate also participates in the course of many chronic neurodegenerative diseases (18). 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 (55).

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 (34). The glutamatergic metabolism, glial proliferation, glial hypertrophy, or activation might be factors contributing to intense neuropathic pain after SCI (34). 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 (56). 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.

Conclusion

In this report, the metabolite levels were almost similar in every cerebral region during the

410 different stages of SCI, but the metabolic kinetics (^{13}C fractional enrichment in different carbon
411 positions of metabolites) were significantly lower in most regions, especially the frontal cortex,
412 parietal cortex, hippocampus, thalamus, and hypothalamus in both acute and chronic SCI groups. After
413 long-term recovery, some metabolic kinetics were recovered, especially in the temporal cortex,
414 occipital cortex and medulla. Furthermore, the impact of SCI on excitatory transmitters (Glu) was
415 greater than on inhibitory neurotransmitters (GABA) in most brain regions, especially for the acute
416 SCI group. The changes in metabolic kinetics revealed that the alteration in metabolism and
417 neurotransmission in different brain regions could present evidence for the alternation of brain glucose
418 oxidation after SCI. Therefore, SCI significantly influenced the cerebral function, especially for acute
419 intervention.
420
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428 The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or
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430
431 **Authors' 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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Figure legends:

Fig. 1: Assessment of motor recovery on chronic SCI rats (n=12 for every group) assessed by BBB scores over four weeks. *Note: The scores indicate that the chronic SCI group showed significant improvement over the Sham group (p=0.0071). Statistical analysis was performed with one-way ANOVA involving multiple comparisons, **p <0.01; Different lowercases mean there was significant difference among different period comparisons in the chronic SCI treatment.*

Fig. 2: HE and Nissl staining of the spinal cord (horizontal and transverse sections) after SCI.

Fig. 3: NMR spectra for total metabolites ($^{12}C+^{13}C$, upper) and ^{13}C related metabolites ($2*^{13}C$, lower) from the POCE (^{1}H observed/^{13}C edited) NMR spectra for the frontal cortex. *Note: subscript: proton signal connected with the ^{13}C position in the metabolites; Asp: Aspartate; Gln: glutamine; Glu: glutamate; Glx: glutamine + glutamate; GABA: γ-aminobutyric acid; Cre: Creatine; NAA: N-acetylaspartate.*

Fig. 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). *Note: (A): Glutamate, (B): GABA, (C): Aspartic acid; Values represent mean ± SEM; *p <0.05, **p <0.01.*

Fig. 5: The ^{13}C fractional enrichments in different kinds of metabolites from [1-^{13}C] glucose in the frontal cortex (A) and temporal cortex (B) for four different groups. *Note: Values represent mean±SEM.*p <0.05, **p <0.01; C2-C4: proton signals connected with the related ^{13}C positions (2-4) in the metabolites.*

Fig. 6: The ^{13}C fractional enrichments in different kinds of metabolites from [1-^{13}C] glucose in the thalamus (A) and hippocampus (B) for four different groups. *Note: Values represent mean ± SEM.*p <0.05, **p <0.01; C2-C4: proton signals connected with the related ^{13}C positions (2-4) in the metabolites.*