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

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<http://dx.doi.org/10.1096/fj.202000447R>

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29 **Nonstandard abbreviations list:** SCI: Spinal cord injury; BBB: Basso, Beatti and Bresnahan; Gln:
30 Glutamine; Glu: Glutamate; Asp: Aspartic acid; NAA: N-acetyl-aspartic acid, Cr: Creatine; GABA:
31 γ -aminobutyric acid; POCE: ^1H observed/ ^{13}C -edited; NMR: Nuclear magnetic resonance; CE:
32 Cerebellum; MED: Medulla; MID: Midbrain; THA: Thalamus; HYP: Hypothalamus; HP:
33 Hippocampus; STR: Striatum; FC: Frontal cortex; OC: Occipital cortex; PC: Parietal cortex; TC:
34 Temporal cortex; PQN: Probabilistic quotient normalization.
35

36 **Abstract**

37 Spinal cord injury (SCI) is a common cause of disability, which often leads to sensorimotor cortex
38 dysfunction above the spinal injury site. However, the cerebral regional effects on metabolic
39 information after SCI have been little studied. Here, adult Sprague-Dawley rats were divided into acute
40 and chronic treatment groups and sham groups with day-matched periods. The BBB (Basso, Beatti
41 and Bresnahan) scores method was utilized to evaluate the changes in behaviors during the recovery
42 of the animals, and the metabolic information was measured with the ¹H-observed/¹³C-edited NMR
43 method. Total metabolic concentrations in every region were almost similar in both treated groups.
44 However, the metabolic kinetics in most regions in the acute group were significantly altered ($p < 0.05$),
45 particularly in the cortical area, thalamus and hippocampus ($p < 0.01$). After long-term recovery, some
46 metabolic kinetics were recovered, especially in the temporal cortex, occipital cortex and medulla.
47 The metabolic kinetic changes revealed the alteration of metabolism and neurotransmission in
48 different brain regions after SCI, which present evidence for the alternation of brain glucose oxidation.
49 Therefore, this shows the significant influence of SCI on cerebral function and neuroscience research.
50 This study also provides the theoretical basis for clinical therapy after SCI, such as mitochondrial
51 transplantation.

52

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

54 **Introduction**

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

65 In recent years, there has been increasing evidence that SCI leads to alterations in brain structure,
66 function and metabolite, by direct effects of nerve damage, secondary mechanisms, and also by longer
67 term injury consequences such as paralysis and neuropathic pain (4). Several studies have shown that
68 SCI resulted in central nervous system injury and structural reorganization of the spine and brain (5,
69 6). Cortical functional reorganization in the sensorimotor areas have been demonstrated in the later
70 stages of SCI (7, 8). In addition, the deafferentation and loss of sensorimotor function after SCI not
71 only directly impacts the sensorimotor system, but also influences other regions, such as the insular,
72 cerebellar, medial prefrontal, anterior cingulate and temporal cortices, which are crucial for processing
73 emotional information and modulating attentional states (9). SCI also induces multiple disturbances
74 in the metabolic network, including oxidative stress, glycolysis, amino acid and lipid metabolism (10,
75 11). Rapid release of excessive glutamate and other neurotransmitters that may directly contribute to

76 cellular damage has been observed following SCI (12). However, previous studies have mainly
77 focused on the SCI site or local area, hence the cerebral regional effects on metabolic information after
78 SCI have been little studied, such as metabolite concentrations and metabolic kinetics of
79 neurotransmitters and some other energetically related neurochemicals.

80 After SCI, mitochondria dysfunction occurred in the brain during the acute phase, followed by
81 inflammatory response and ER stress aroused at subacute phase (13). While normal brain function
82 requires a stable energy supply, disturbances in brain energy metabolism have been associated with
83 neurological dysfunctions and cognitive impairment (14). Glucose is considered as the main substrate
84 for neuronal energy metabolism in the mammalian brain (15). It has also been estimated that the
85 cycling between glutamine (Gln) and glutamate (Glu) accounts for more than 80% of cerebral glucose
86 consumption (16). The tight coupling between the Glu–Gln cycle and brain energetics is largely tied
87 to the nearly 1:1 stoichiometry between glucose oxidation and the rate of astrocytic Glu uptake (17).
88 Thus, it is valuable to investigate the imbalance of brain energy metabolism in neurons and astrocytes
89 and explore the pathogenesis of neurological disorders after SCI (18).

90 ¹H observed/¹³C-edited (POCE) nuclear magnetic resonance (NMR) technique is a promising
91 approach for investigating the metabolic kinetics in astrocytes, specific neurons and their interactions
92 (19). Metabolic information between neuronal and astrocytic interaction can be investigated by the
93 POCE method combined with the infusion of ¹³C-labeled glucose/acetate (20-22). We hypothesized
94 that SCI could produce lasting deficits in brain metabolism. Thus, the purpose of the present study
95 was to evaluate the effect of SCI on regional metabolic concentrations and rates of turnover of
96 glutamate, glutamine, and GABA (γ-aminobutyric acid) and other metabolites in the rat brain. The
97 changes in metabolic information could reveal the influence on different brain regions, which could

98 present evidence for the alternation of regional cerebral glucose oxidation and cerebral function after
99 SCI. Furthermore, this study could provide the theoretical basis for clinical therapy after SCI, such as
100 mitochondrial transplantation.

101

102 **Methods**

103 *Animals*

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

112 In the current study, 52 female adult Sprague-Dawley rats (n=12 for each group in the NMR study,
113 and n=4 for the histology study) were ordered from VITAL RIVER (Beijing, China) and kept in SPF
114 (Specific pathogen Free) animal residence (Wuhan, China). Rats were housed in plastic cages (three
115 animals per cage) in a climate-controlled room with 12 h of light-dark illumination cycle at 25±1 °C
116 and relative 50 ± 10% humidity. During the experiment, all rats were allowed free access to laboratory
117 standard food (Product No: 190011304, WQJX Biotech, Wuhan, China) and water. Due to failure
118 related to animal surgery (n=3) and tail vein catheterization (n=4), seven of the 48 rats were not
119 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

143 allowed to move freely and scored for their ability to use their hindlimbs. A 21- point BBB locomotion
144 scale was used based on the movement of joints, placement of paws and coordination of forepaw and
145 hind limbs (26). The BBB scores were determined 0, 1, 2, 3 and 4 weeks post-SCI to assess recovery
146 of locomotion in the chronic SCI treatment group.

147

148 *Perfusion and histology of the spinal cord*

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

156

157 *Infusion techniques*

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

162 On the experimental day, rats were initially anesthetized with 4.0-5.0% isoflurane mixed with air,
163 and 1.5-2.5% isoflurane to maintain the anesthesia state. The adequate level of anesthesia was verified
164 by a lack of withdrawal response to a foot pinch. Then, one lateral tail vein was catheterized with PE50
165 tubing (Instech, PA, USA) for the infusion of [1-¹³C] glucose, and the tube was immobilized to the tail
166 with adhesive paper tape. Then those animals were recovered for about 15 minutes until they showed
167 free movement and normal grooming. The infusion line was connected to a swivel (Instech, PA, USA)

168 and suspended from the center of the cage to avoid entanglement of the line during the rat movement.
169 The other end of the swivel was connected to the infusion pump (Fusion100, Chemyx, TX, USA) with
170 PE50 tubing. After everything was set up, the animal was allowed to recover for another 15 min. Then,
171 [$1\text{-}^{13}\text{C}$] glucose was infused through the lateral tail vein following a former infusion protocol (22) and
172 the infusion ceased after 20 minutes. During the whole procedure, the rat had freedom of movement
173 in the cage (22). All animals were sacrificed by the head-focused microwave irradiation method (1kW,
174 Tangshan Nanosource Microwave Thermal Instrument Manufacturing Co. Ltd., Heibei, P.R. China).
175 Then a blood sample (~1 mL) was withdrawn and the brain was manually dissected into 11 different
176 regions as described previously (22): cerebellum (CE), medulla (MED), midbrain (MID), thalamus
177 (THA), hypothalamus (HYP), hippocampus (HP), striatum (STR), frontal cortex (FC), occipital cortex
178 (OC), parietal cortex (PC), and temporal cortex (TC). The tissue was weighed, frozen in liquid nitrogen,
179 and stored at $-80\text{ }^{\circ}\text{C}$ until further processing. Four rats failed due to very low [$1\text{-}^{13}\text{C}$] glucose
180 enrichment (<10%), caused by the failure of tail vein catheterization. Thus, the cerebral data from
181 these animals were ignored. Therefore, there were 41 rats that successfully completed the study,
182 including 20 rats from the two separate control groups (10 by 10) and 21 rats from the two SCI groups
183 (9 for acute SCI and 12 for Chronic SCI).

184

185 *Sample collection and preparation*

186 The preparation of brain tissue extracts was conducted using the same methanol-ethanol
187 extraction method which was described in our previous work (27). Briefly, HCl/methanol (80 μL , 0.1
188 M) was added to the brain samples, and the tissues were initially homogenized with TissueLyser
189 (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 (600 μL D_2O with 0.2 M
196 $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$, 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 ^1H -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, ^1H - ^{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, $^{12}\text{C}+^{13}\text{C}$), 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

212 *NMR Spectra Processing*

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

217 *Relative concentration calculation*

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

229 *Metabolic enrichment calculation*

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

234 used for the analysis.

235 *Data analysis*

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

241

242 **Results**

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

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

251 *SCI leads to neurons death and glial cells activation*

252 In the sham group, the gray and white matter structures of the spinal cord are clear, the cells are
253 evenly distributed and orderly arranged, neurons and glial cells are clearly visible, and no cavities and
254 necrotic tissues are observable (Fig. 2). In the acute SCI group, the structure of the injury site is
255 disordered, and the boundary between the gray matter and white matter of the spinal cord is unclear.

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

259 *POCE NMR Spectrum of brain extracts*

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

267 *Metabolites concentration in different brain regions after SCI*

268 To explore the changes of metabolites in different brain regions, which might be related to
269 neurobehavioral abnormalities after SCI, the relative concentrations of metabolites in four different
270 groups (Acute SCI vs. Sham (Acute); Chronic SCI vs. Sham (Chronic)) were compared among 11
271 brain regions. After comparison, the concentrations of most metabolites did not change in these two
272 pairs. For example, several metabolite concentrations (glutamate, GABA and aspartic acid (Asp)) are
273 illustrated in Fig. 4. For these metabolites, there were only a few changes among 22 pair comparisons
274 in each metabolite (two group pair × 11 brain regions). Glutamate was significantly decreased only in
275 FC for the chronic SCI group (Fig. 4A, $p=0.002$). However, there were opposite changes observed for
276 GABA in the cortex and deep brain areas, and GABA was increased in HYP ($p=0.008$) and TC
277 ($p=0.035$, 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 $[1-^{13}\text{C}]$ -glucose infusion.

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

283 With the infusion of $[1-^{13}\text{C}]$ -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, Glu₄ (glutamatergic neuron) and GABA₂ (GABAergic neuron) were
286 labeled with ^{13}C probe, and Gln₄ 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 $[1-^{13}\text{C}]$ glucose in the prefrontal cortex of
296 different groups exhibited significant changes. The ^{13}C enrichments in Glu₄ ($p=0.037$), Asp₃ ($p=0.028$),
297 GABA₃ ($p=0.048$) and Glx₃ ($p=0.046$) in the acute SCI group were found to be significantly lower
298 than in the acute sham group. In addition, Glu₄ ($p=0.011$), Asp₃ ($p=0.001$), Glu₃ ($p=0.023$), GABA₃
299 ($p=0.016$), Glx₂ ($p=0.023$) 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 Glu₄ ($p=0.029$), GABA₃ ($p=0.035$),
302 Asp₃ ($p=0.004$), Glu₃ ($p=0.016$), Glx₃ ($p=0.022$) and Glx₂ ($p=0.026$), and only Glu₄ ($p=0.043$) 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 ¹³C enrichment in different
305 types of metabolites in the chronic SCI group, such as Glu₄ ($p=0.005$), GABA₄ ($p=0.005$), Asp₃
306 ($p=0.001$), GABA₃ ($p=0.033$), Glx₃ ($p=0.018$), Glx₂ ($p=0.011$) and Ala₃ ($p=0.01$). However, only Glu₄
307 ($p=0.047$) and Gln₄ ($p=0.047$) were decreased in the acute SCI group (Fig. 6A). The enrichments of
308 Glu₄ and Gln₄ 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 ¹H-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 ¹H-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,

344 mitochondrial transplantation is effective in promoting recovery after ischemic injury to cardiac tissue
345 (41). Many pharmacological agents that have proven beneficial for the treatment of SCI *in vivo* to
346 some extent affect mitochondria or mitochondrial function (39). For example, the antibiotic
347 minocycline was found to have neuroprotective effects and induced behavioral and cellular recovery
348 after SCI in rats (42). NACA treatment significantly maintained acute mitochondrial bioenergetics and
349 normalized GSH levels following SCI, and the prolonged delivery resulted in significant tissue sparing
350 and improved recovery of hindlimb function (43).

351

352 *Regional effects and SCI*

353 The metabolic information, required to determine metabolic fluxes, commonly vary with cerebral
354 regions as shown in the human (44), rat (45) and mouse (46) brains. Both the cerebral cortex and deep
355 brain regions have their own variation characteristics after SCI. Comparisons of metabolic enrichment
356 in the cerebral cortex, thalamus and hippocampus revealed substantial and highly significant regional
357 variations (Fig. 5 and 6).

358 In previous studies, the cerebral cortex was selected as a whole region in order to compare
359 regional changes in metabolite concentrations (45). However, the regional cerebral effects on
360 metabolic information in the brain cortex after SCI have been little studied. The present study revealed
361 changes in different brain cortices and showed distinct variations of metabolic kinetics in the cortex.
362 The frontal cortex showed that the ¹³C enrichment in neurotransmitters were markedly decreased in
363 both chronic and acute groups. However, the impact of SCI on the TC (Fig. 5B) and OC (Fig. S1B)
364 were mainly significantly decreased in the acute group (Fig. 5), and the alterations were mostly
365 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 ¹³C enrichment amino acids in the thalamus mainly decreased in the chronic group, while
382 only Glu₄ and Gln₄ 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

388 alterations -Fig. 5, 6 and S1: 9 regions for Glu₄ and 2 regions for GABA₂). Glutamate and glutamine
389 are relatively abundant amino acids in the brain that are critical for neuronal function (54), and they
390 were involved in the regulation of brain energy metabolism (18). A dysfunction in the homeostasis,
391 recycling, and metabolism of glutamate also participates in the course of many chronic
392 neurodegenerative diseases (18). Alteration of this cyclic nature of Glu and Gln has been shown to
393 play an important role in the regulation of various neurological disorders, including epilepsy, multiple
394 sclerosis, traumatic brain injury, schizophrenia, and brain tumors (55).

395 Neuropathic pain after SCI was also reported to be always associated with altered thalamic
396 anatomy, biochemistry, and activity, which may result in disturbed thalamocortical circuits (34). The
397 glutamatergic metabolism, glial proliferation, glial hypertrophy, or activation might be factors
398 contributing to intense neuropathic pain after SCI (34). There is no doubt that unbalanced metabolism
399 of neurotransmitters may be involved in the process of neuropathic pain. Specifically, it has been
400 shown that neuropathic pain after SCI is associated with changes in thalamic neurons, which
401 subsequently may make these neurons hyperexcitable, and as such, may act as a pain generator or
402 amplifier (56). Hyperexcitability also plays a role in the genesis of multi-sensory symptoms after SCI,
403 this might either be a common phenomenon across different sensory cortices, or one might postulate
404 hyperexcitability within a structure with sensory input to the thalamus. Thus, the current study
405 speculates that the glutamatergic activity could be related to neuropathic pain caused by SCI, which
406 could provide an avenue for the clinical therapy of SCI.

407

408 **Conclusion**

409 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

421 **Acknowledgement**

422 This work was supported by grants from Key Research Projects of Ningxia Hui Autonomous
423 Region (2018BCG01002), the National Natural Science Foundation of China (31771193, 81760449),
424 the Natural Science Foundation of Ningxia (NZ16127) and the Youth Innovation Promotion
425 Association of Chinese Academy of Sciences (Y6Y0021004).

426

427 **Declaration of conflicting interests**

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

430

431 **Authors' contributions**

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

436 **Data availability statement**

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

438

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626

627

628 **Figure legends:**

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

634

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

636

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

642

643 **Fig. 4:** The relative concentrations of metabolites in 11 different brain regions for four different groups
644 (Sham (Acute):12; Acute SCI: 11; Sham (Chronic): 10; and Chronic SCI: 12). *Note: (A): Glutamate,*
645 *(B): GABA, (C): Aspartic acid; Values represent mean \pm SEM; $*p < 0.05$, $**p < 0.01$.*

646

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

651

652 **Fig. 6:** The ^{13}C fractional enrichments in different kinds of metabolites from $[1-^{13}\text{C}]$ glucose in the
653 thalamus (A) and hippocampus (B) for four different groups. *Note: Values represent mean \pm SEM. $*p$*
654 *< 0.05 , $**p < 0.01$; C2-C4: proton signals connected with the related ^{13}C positions (2-4) in the*
655 *metabolites.*