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

cord injury

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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: ^{1}H 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

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

54

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   [1]H observed/^{13}C-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 ^{13}C-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 messaged 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 ℃

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.

Animal experiment

At first, all animals were randomly divided into four equal groups: the acute SCI treatment group (three 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.*, 5mg/100g). 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 to expose the intact dura. The animal was transferred to beneath the IH impactor which was equipped with a 4 mm tip, and the incision site was centered. 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 h after SCI. Penicillin was continuously injected at 100000 units/time/day up to 7 days. The bladder was massaged 2-3 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.

Basso, Beattie, and Bresnahan (BBB) 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

7

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 transcardially 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 ^{13}C enrichment into different carbon positions of

159 metabolites after the infusion of [1-^{13}C] glucose. For this method, the higher enrichment of [1-^{13}C]

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-^{13}C] 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-^{13}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℃ until further processing. Four rats failed due to very low [1-^{13}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 (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

212 *NMR Spectra Processing*

213   All FID signals of ^{1}H-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]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]C +[13]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

11

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 ± 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 ^{13}C 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}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

14

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,

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 ^{13}C 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,

17

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

18

388 alterations -Fig. 5, 6 and S1: 9 regions for Glu_4 and 2 regions for $GABA_2$). 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

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

439   **References**

440 1. Ahuja, C. S., Wilson, J. R., Nori, S., Kotter, M. R. N., Druschel, C., Curt, A., and
441   Fehlings, M. G. (2017) Traumatic spinal cord injury. *Nat Rev Dis Primers* **3**,
442   17018
443 2. Oyinbo, C. A. (2011) Secondary injury mechanisms in traumatic spinal cord
444   injury: a nugget of this multiply cascade. *Acta Neurobiol Exp* **71**, 281-299
445 3. Frigon, A., and Rossignol, S. (2006) Functional plasticity following spinal cord
446   lesions. *Progress in Brain Research* **157**, 231-260
447 4. Solstrand Dahlberg, L., Becerra, L., Borsook, D., and Linnman, C. (2018) Brain
448   changes after spinal cord injury, a quantitative meta-analysis and review.
449   *Neurosci Biobehav Rev* **90**, 272-293
450 5. Freund, P., Weiskopf, N., Ashburner, J., Wolf, K., Sutter, R., Altmann, D. R.,
451   Friston, K., Thompson, A., and Curt, A. (2013) MRI investigation of the
452   sensorimotor cortex and the corticospinal tract after acute spinal cord injury:
453   a prospective longitudinal study. *Lancet Neurol* **12**, 873-881
454 6. Grabher, P., Callaghan, M. F., Ashburner, J., Weiskopf, N., Thompson, A. J., Curt,
455   A., and Freund, P. (2015) Tracking sensory system atrophy and outcome
456   prediction in spinal cord injury. *Ann Neurol* **78**, 751-761
457 7. Jurkiewicz, M. T., Mikulis, D. J., McIlroy, W. E., Fehlings, M. G., and Verrier, M. C.
458   (2007) Sensorimotor cortical plasticity during recovery following spinal cord
459   injury: a longitudinal fMRI study. *Neurorehabil Neural Repair* **21**, 527-538
460 8. Henderson, L. A., Gustin, S. M., Macey, P. M., Wrigley, P. J., and Siddall, P. J. (2011)
461   Functional reorganization of the brain in humans following spinal cord injury:
462   evidence for underlying changes in cortical anatomy. *J Neurosci* **31**, 2630-
463   2637
464 9. Wrigley, P. J., Gustin, S. M., Macey, P. M., Nash, P. G., Gandevia, S. C., Macefield,
465   V. G., Siddall, P. J., and Henderson, L. A. (2009) Anatomical changes in human
466   motor cortex and motor pathways following complete thoracic spinal cord
467   injury. *Cereb Cortex* **19**, 224-232

468   10.   Long, Y. C., Kostovski, E., Boon, H., Hjeltnes, N., Krook, A., and Widegren, U.
469   (2011) Differential expression of metabolic genes essential for glucose and
470   lipid metabolism in skeletal muscle from spinal cord injured subjects. *J Appl*
471   *Physiol* **110**, 1204-1210

472   11.   Dulin, J. N., Karoly, E. D., Wang, Y., Strobel, H. W., and Grill, R. J. (2013)
473   Licofelone modulates neuroinflammation and attenuates mechanical
474   hypersensitivity in the chronic phase of spinal cord injury. *J Neurosci* **33**, 652-
475   664

476   12.   Vera-Portocarrero, L. P., Mills, C. D., Ye, Z., Fullwood, S. D., McAdoo, D. J.,
477   Hulsebosch, C. E., and Westlund, K. N. (2002) Rapid changes in expression of
478   glutamate transporters after spinal cord injury. *Brain Res* **927**, 104-110

479   13.   Baek, A., Cho, S. R., and Kim, S. H. (2017) Elucidation of Gene Expression
480   Patterns in the Brain after Spinal Cord Injury. *Cell Transplant* **26**, 1286-1300

481   14.   Lyons, D. N., Vekaria, H., Macheda, T., Bakshi, V., Powell, D. K., Gold, B. T., Lin, A.
482   L., Sullivan, P. G., and Bachstetter, A. D. (2018) A Mild Traumatic Brain Injury
483   in Mice Produces Lasting Deficits in Brain Metabolism. *J Neurotrauma* **35**,
484   2435-2447

485   15.   Siesjo, B. K. (1978) Brain energy metabolism and catecholaminergic activity
486   in hypoxia, hypercapnia and ischemia. *J Neural Transm Suppl* **14**, 17-22

487   16.   Sibson, N. R., Dhankhar, A., Mason, G. F., Rothman, D. L., Behar, K. L., and
488   Shulman, R. G. (1998) Stoichiometric coupling of brain glucose metabolism
489   and glutamatergic neuronal activity. *Proc Natl Acad Sci U S A* **95**, 316-321

490   17.   Ramadan, S., Lin, A., and Stanwell, P. (2013) Glutamate and glutamine: a
491   review of in vivo MRS in the human brain. *Nmr Biomed* **26**, 1630-1646

492   18.   Escartin, C., Valette, J., Lebon, V., and Bonvento, G. (2006) Neuron-astrocyte
493   interactions in the regulation of brain energy metabolism: a focus on NMR
494   spectroscopy. *J Neurochem* **99**, 393-401

495   19.   Sonnewald, U., and Kondziella, D. (2003) Neuronal glial interaction in
496   different neurological diseases studied by ex vivo 13C NMR spectroscopy.
497   *Nmr Biomed* **16**, 424-429

498   20.   Deelchand, D. K., Nelson, C., Shestov, A. A., Ugurbil, K., and Henry, P. G. (2009)
499   Simultaneous measurement of neuronal and glial metabolism in rat brain in
500   vivo using co-infusion of [1,6-13C2]glucose and [1,2-13C2]acetate. *J Magn*
501   *Reson* **196**, 157-163

502   21.   de Graaf, R. A., Mason, G. F., Patel, A. B., Behar, K. L., and Rothman, D. L. (2003)
503   In vivo ^{1}H-[^{13}C]-NMR spectroscopy of cerebral metabolism. *Nmr Biomed* **16**,
504   339-357

505   22.   Wang, J., Jiang, L. H., Jiang, Y. F., Ma, X. X., Chowdhury, G. M. I., and Mason, G. F.
506   (2010) Regional metabolite levels and turnover in the awake rat brain under
507   the influence of nicotine. *J Neurochem* **113**, 1447-1458

508   23.   Burnside, E. R., De Winter, F., Didangelos, A., James, N. D., Andreica, E. C.,
509   Layard-Horsfall, H., Muir, E. M., Verhaagen, J., and Bradbury, E. J. (2018)
510   Immune-evasive gene switch enables regulated delivery of chondroitinase
511   after spinal cord injury. *Brain* **141**, 2362-2381

512 24. Manohar, A., Foffani, G., Ganzer, P. D., Bethea, J. R., and Moxon, K. A. (2017)
513   Cortex-dependent recovery of unassisted hindlimb locomotion after
514   complete spinal cord injury in adult rats. *Elife* **6**, e23532

515 25. Bonizzato, M., Pidpruzhnykova, G., DiGiovanna, J., Shkorbatova, P., Pavlova, N.,
516   Micera, S., and Courtine, G. (2018) Brain-controlled modulation of spinal
517   circuits improves recovery from spinal cord injury. *Nat Commun* **9**, 3015

518 26. Basso, D. M., Beattie, M. S., and Bresnahan, J. C. (1995) A Sensitive and Reliable
519   Locomotor Rating-Scale for Open-Field Testing in Rats. *J Neurotraum* **12**, 1-
520   21

521 27. Liu, T., He, Z., Tian, X., Kamal, G. M., Li, Z., Liu, Z., Liu, H., Xu, F., Wang, J., and
522   Xiang, H. (2017) Specific patterns of spinal metabolites underlying alpha-Me-
523   5-HT-evoked pruritus compared with histamine and capsaicin assessed by
524   proton nuclear magnetic resonance spectroscopy. *Biochim Biophys Acta Mol*
525   *Basis Dis* **1863**, 1222-1230

526 28. Liu, Y., Cheng, J., Liu, H. L., Deng, Y. H., Wang, J., and Xu, F. Q. (2017) NMRSpec:
527   An integrated software package for processing and analyzing one
528   dimensional nuclear magnetic resonance spectra. *Chemometr Intell Lab* **162**,
529   142-148

530 29. Dieterle, F., Ross, A., Schlotterbeck, G., and Senn, H. (2006) Probabilistic
531   quotient normalization as robust method to account for dilution of complex
532   biological mixtures. Application in H-1 NMR metabonomics. *Anal Chem* **78**,
533   4281-4290

534 30. Huo, Z., Yu, L., Yang, J., Zhu, Y., Bennett, D. A., and Zhao, J. (2020) Brain and
535   blood metabolome for Alzheimer's dementia: findings from a targeted
536   metabolomics analysis. *Neurobiology of Aging* **86**, 123-133

537 31. Jacyna, J., Wawrzyniak, R., Balayssac, S., Gilard, V., Malet-Martino, M., Sawicka,
538   A., Kordalewska, M., Nowicki, Ł., Kurek, E., Bulska, E., Patejko, M.,
539   Markuszewski, M., Gutknecht, P., Matuszewski, M., Siebert, J., Kaliszan, R., and
540   Markuszewski, M. J. (2019) Urinary metabolomic signature of muscle-
541   invasive bladder cancer: A multiplatform approach. *Talanta* **202**, 572-579

542 32. Tam, S., Barry, R. L., Bartha, R., and Duggal, N. (2010) Changes in functional
543   magnetic resonance imaging cortical activation after decompression of
544   cervical spondylosis: case report. *Neurosurgery* **67**, E863-E864

545 33. Likavcanova, K., Urdzikova, L., Hajek, M., and Sykova, E. (2008) Metabolic
546   changes in the thalamus after spinal cord injury followed by proton MR
547   spectroscopy. *Magn Reson Med* **59**, 499-506

548 34. Gustin, S. M., Wrigley, P. J., Youssef, A. M., McIndoe, L., Wilcox, S. L., Rae, C. D.,
549   Edden, R. A., Siddall, P. J., and Henderson, L. A. (2014) Thalamic activity and
550   biochemical changes in individuals with neuropathic pain after spinal cord
551   injury. *Pain* **155**, 1027-1036

552 35. Martinez-Reyes, I., and Chandel, N. S. (2020) Mitochondrial TCA cycle
553   metabolites control physiology and disease. *Nat Commun* **11**, 102

554 36. Patel, S. P., Sullivan, P. G., Lyttle, T. S., and Rabchevsky, A. G. (2010) Acetyl-L-
555   carnitine ameliorates mitochondrial dysfunction following contusion spinal

556   cord injury. *J Neurochem* **114**, 291-301

557   37.   Patel, S. P., Sullivan, P. G., Pandya, J. D., and Rabchevsky, A. G. (2009)
558   Differential effects of the mitochondrial uncoupling agent, 2,4-dinitrophenol,
559   or the nitroxide antioxidant, Tempol, on synaptic or nonsynaptic
560   mitochondria after spinal cord injury. *J Neurosci Res* **87**, 130-140

561   38.   Gollihue, J. L., Patel, S. P., Eldahan, K. C., Cox, D. H., Donahue, R. R., Taylor, B. K.,
562   Sullivan, P. G., and Rabchevsky, A. G. (2018) Effects of Mitochondrial
563   Transplantation on Bioenergetics, Cellular Incorporation, and Functional
564   Recovery after Spinal Cord Injury. *J Neurotrauma* **35**, 1800-1818

565   39.   Scholpa, N. E., and Schnellmann, R. G. (2017) Mitochondrial-Based
566   Therapeutics for the Treatment of Spinal Cord Injury: Mitochondrial
567   Biogenesis as a Potential Pharmacological Target. *J Pharmacol Exp Ther* **363**,
568   303-313

569   40.   McCully, J. D., Cowan, D. B., Emani, S. M., and Del Nido, P. J. (2017)
570   Mitochondrial transplantation: From animal models to clinical use in humans.
571   *Mitochondrion* **34**, 127-134

572   41.   Pacak, C. A., Preble, J. M., Kondo, H., Seibel, P., Levitsky, S., Del Nido, P. J., Cowan,
573   D. B., and McCully, J. D. (2015) Actin-dependent mitochondrial internalization
574   in cardiomyocytes: evidence for rescue of mitochondrial function. *Biol Open*
575   **4**, 622-626

576   42.   Ahmad, M., Zakaria, A., and Almutairi, K. M. (2016) Effectiveness of
577   minocycline and FK506 alone and in combination on enhanced behavioral
578   and biochemical recovery from spinal cord injury in rats. *Pharmacol Biochem*
579   *Behav* **145**, 45-54

580   43.   Patel, S. P., Sullivan, P. G., Pandya, J. D., Goldstein, G. A., VanRooyen, J. L., Yonutas,
581   H. M., Eldahan, K. C., Morehouse, J., Magnuson, D. S., and Rabchevsky, A. G.
582   (2014) N-acetylcysteine amide preserves mitochondrial bioenergetics and
583   improves functional recovery following spinal trauma. *Exp Neurol* **257**, 95-
584   105

585   44.   Emir, U. E., Auerbach, E. J., Van De Moortele, P. F., Marjanska, M., Ugurbil, K.,
586   Terpstra, M., Tkac, I., and Oz, G. (2012) Regional neurochemical profiles in the
587   human brain measured by (1)H MRS at 7 T using local B(1) shimming. *Nmr*
588   *Biomed* **25**, 152-160

589   45.   Tkac, I., Rao, R., Georgieff, M. K., and Gruetter, R. (2003) Developmental and
590   regional changes in the neurochemical profile of the rat brain determined by
591   in vivo 1H NMR spectroscopy. *Magn Reson Med* **50**, 24-32

592   46.   Liu, T., Li, Z., He, J., Yang, N., Han, D., Li, Y., Tian, X., Liu, H., Manyande, A., Xiang,
593   H., Xu, F., Wang, J., and Guo, X. (2020) Regional Metabolic Patterns of
594   Abnormal Postoperative Behavioral Performance in Aged Mice Assessed by
595   ^{1}H-NMR Dynamic Mapping Method. *Neuroscience Bulletin* **36**, 25-38

596   47.   Scandola, M., Dodoni, L., Lazzeri, G., Arcangeli, C. A., Avesani, R., Moro, V., and
597   Ionta, S. (2019) Neurocognitive Benefits of Physiotherapy for Spinal Cord
598   Injury. *Journal of Neurotrauma* **36**, 2028-2035

599   48.   Perruchoud, D., Pisotta, I., Carda, S., Murray, M. M., and Ionta, S. (2016)

Biomimetic rehabilitation engineering: the importance of somatosensory feedback for brain-machine interfaces. *J Neural Eng* **13**, 041001

49. Humanes-Valera, D., Aguilar, J., and Foffani, G. (2013) Reorganization of the intact somatosensory cortex immediately after spinal cord injury. *PLoS One* **8**, e69655

50. Endo, T., Spenger, C., Tominaga, T., Brene, S., and Olson, L. (2007) Cortical sensory map rearrangement after spinal cord injury: fMRI responses linked to Nogo signalling. *Brain* **130**, 2951-2961

51. Hirata, A., Aguilar, J., and Castro-Alamancos, M. A. (2009) Influence of subcortical inhibition on barrel cortex receptive fields. *J Neurophysiol* **102**, 437-450

52. Magon, S., May, A., Stankewitz, A., Goadsby, P. J., Tso, A. R., Ashina, M., Amin, F. M., Seifert, C. L., Chakravarty, M. M., Muller, J., and Sprenger, T. (2015) Morphological Abnormalities of Thalamic Subnuclei in Migraine: A Multicenter MRI Study at 3 Tesla. *J Neurosci* **35**, 13800-13806

53. Lenz, F. A., Tasker, R. R., Dostrovsky, J. O., Kwan, H. C., Gorecki, J., Hirayama, T., and Murphy, J. T. (1987) Abnormal single-unit activity recorded in the somatosensory thalamus of a quadriplegic patient with central pain. *Pain* **31**, 225-236

54. Erecinska, M., and Silver, I. A. (1990) Metabolism and role of glutamate in mammalian brain. *Prog Neurobiol* **35**, 245-296

55. Goryawala, M. Z., Sheriff, S., and Maudsley, A. A. (2016) Regional distributions of brain glutamate and glutamine in normal subjects. *Nmr Biomed* **29**, 1108-1116

56. Waxman, S. G., and Hains, B. C. (2006) Fire and phantoms after spinal cord injury: Na+ channels and central pain. *Trends Neurosci* **29**, 207-215

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.*