Anatomical mechanism of spontaneous recovery in regions caudal to thoracic spinal cord injury lesions in rats

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Background: The nerve fibre circuits around a lesion play a major role in the spontaneous recovery process after spinal cord hemisection in rats. The aim of the present study is: in the re–control process, do all spinal cord nerves below the lesion site participate , or do the spinal cord nerves of only one vertebral segment have a role in repair? **Methods:** First we made a T7 spinal cord hemisection in 50 rats. Eight weeks later, they were divided into 3 groups based on distinct second operations at T7: ipsilateral hemisection operation, contralateral hemisection, or transection. We then tested recovery of hindlimbs for another 8 weeks. The first step was to confirm the lesion had role or not in the spontaneous recovery process. Secondly, we performed T7 spinal cord hemisections in 125 rats. Eight weeks later, we performed a second single hemisection on the ipsilateral side at T8-T12 and then tested hindlimb recovery for another 6 weeks. **Results:** In the first part, the Basso, Beattie, Bresnahan (BBB) scores and the electrophysiology tests of both hindlimbs weren't significantly different after the second hemisection of the ipsilateral side. In the second part, the closer the second hemisection was to T12, the more substantial the resulting impairment in BBB score tests and prolonged latency periods. **Conclusions:** The nerve regeneration from the lesion area after hemisection has no effect on spontaneous recovery of the spinal cord. Repair is carried out by all vertebrae caudal and ipsilateral to the lesion, with T12 being most important.

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Abstract

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Conclusions: The nerve regeneration from the lesion area after hemisection has no effect on spontaneous recovery of the spinal cord. Repair is carried out by all vertebrae caudal and ipsilateral to the lesion, with T12 being most important.

Keywords: spinal cord injury, hemisection, transection, recovery, electrophysiology, nerve

1 Introduction

Brain is plastic and mammals are capable of spontaneous recovery after spinal cord injury. The mechanisms underlying this process are not yet clear and are disputed. After brain or spinal cord injury, many researchers have found that transplantation of multifunctional three-dimensional scaffolds and stem cells treatment with neurotrophic factors, administration of small molecules, or genetic modifications in the lesion area promote neuronal regeneration in the lesion and improve motor function recovery (Estrada et al. 2014; Fan et al. 2010; Jee et al. 2012; McCall et al. 2012; Shi et al. 2015; Tan et al. 2016; Wright et al. 2011). In addition, several groups have shown that, after hemisection of the thoracic spinal cord, both hindlimbs show significant improvement 3-5 weeks later. Moreover, if a second hemisection on the side contralateral to the lesion was performed, rats showed complete paralysis of both hind limbs with no signs of recovery of locomotor function over 4 weeks. For example, Courtine et al. first produced a left–side hemisection model at thoracic segment 12 (T12 refers to the spinal cord level). Then, 10 weeks later, they performed a second hemisection on the contralateral side (T7). The results showed that the rats initially lost all movement on the T7 side and some of the movement on the T12 side. However, when the rats were subjected to the T7 and T12 hemisection at the same time, both hindlimbs instantly lost all movement (Courtine et al. 2008). This observation demonstrates that nerve fibers around the lesion participate in repair. These fibers must originate rostral to the lesion on the ipsilateral side, then cross the midline to the contralateral side, travel down the spinal cord, and re-cross the midline caudal to the lesion (Ballermann & Fouad 2006; Courtine et al. 2008; Etlin et al. 2010; Reed et al. 2008). These two different repair mechanisms are in conflict with each other and further research is needed to confirm and explain the repair process. If the mechanisms of repair in both contexts were resolved it would inform novel

treatments to promote recovery and further improve limb function in patients with spinal cord injury.

Among the questions that remain to be answered is whether the nerve fiber circuits that control the ipsilateral hindlimb after injury are comprised of spinal cord nerves in a single vertebra or spinal cord nerves in multiple vertebrae contribute to these nerve fiber circuits and participate in the recovery process. Accordingly, we designed a study to resolve these two possibilities. We first carried out a hemisection of thoracic vertebrae 7 (T7) on the left side of spinal cord, then 8 weeks later performed a second hemisection of thoracic vertebrae 8-12 (T8-T12) on the ipsilateral side. We then compared the extent of recovery of hindlimb function acrosss each hemisection group. In this study, abbreviations such as T7 indicate the vertebral segments.

2 Materials and Methods

2.1 Animals

In this study, we used adult Sprague–Dawley rats (200–220 g). All rats were allowed to acclimate to the new environment for 7 days before the start of any experimental procedures. The rats were housed on a 12 h light-dark cycle with food and water provided ad libitum. All rats were deeply anesthetized before any surgical procedures were performed (10% chloral hydrate, 3.5 ml/kg). After surgery, antibiotic (Penicillin, 128000UI/kg) and 10 ml of sterile saline were administered subcutaneously each day during the first week. After each operation, the rat was placed in a separate cage for 7 days before it was housed with other rats. The rats in the study were obtained from the Vital River Company. Ethical approval was obtained from the Beijing Neurosurgical Institute Laboratory Animals Ethics Committee in China.

In the first part of study, there were 50 rats in total and 10 rats died throughout the study, the mortality was 20%. In the second part of study, there were 125 rats in total and 16 rats died throughout the study, the mortality was 12.8%.

2.2 Groups

In the first part of the study, we investigated if regenerated axons coursed ipsilaterally or contralaterally. All rats underwent two operations on T7 spinal cord and were divided into 5 groups (Fig. 1A-E). The first group was a sham operation group (N=10) in which both operations were sham. Groups 2-5 all first received a hemisection of the left side of spinal cord at T7, but had different second operations. The second group was the control group (N=6), in which the second operation was sham, consisted a midline cut in the spinal cord at T7. The third group was the ipsilateral experimental group (N=8), in which a second hemisection was conducted on the ipsilateral side. The fourth group was the contralateral group (N=8), in which a second hemisection was made on the contralateral side. The fifth was the transection group (N=8), which received a full transection at T7 in the second operation.

In the second part of the study, we investigated the innervation of vertebrae downstream of the lesion site to determine if regenerated axons target single or multiple vertebrae. These experiments comprised 3 main groups, each of which were divided into 5 smaller sub-groups defined according to the site of the second operation: T8-T12. The first group was the operation group (N=37, Fig. 2A1-A2), in which all rats underwent 2 hemisection operations. The first operation was at T7 on the left side; 8 weeks later the second operation was carried out ipsilaterally at a single vertebra from T8-T12 depending on sub-group. The second group was the control group (N=37, Fig. 2B1-B5). All received a hemisection at T7 on the left side, and 8

111 weeks later underwent a sham operation at a single vertebra at T8-T12 depending on sub-group.
112 The third was the sham operation group (N=35, Fig. 2C1-C5), in which rats in all sub-groups
113 received a sham operation at both time points.
114
115 **2.3 Hemisection operation**
116 **2.3.1 The first hemisection operation**
117 We generated the first hemisection operation according to previously published methods
118 (Arvanian et al. 2009). In the operation, we used sharp scalpel not scissors to separate along the
119 midline of spinal cord, which might cause less injury to the spinal cord. Before normal bladder
120 control returned, we manually expressed the bladder of each rat once per day.
121 **2.3.2 The second hemisection operation**
122 The second hemisection operation was performed 8 weeks after the first hemisection operation.
123 Except for the vertebra receiving a laminectomy, other operation procedures were the same as in
124 the first hemisection operation.
125
126 **2.4 Basso, Beattie, Bresnahan (BBB) score tests in both hindlimbs**
127 Motor performance was scored were performed according to the well-known Open Field BBB
128 locomotor scale (Basso et al. 1995). It was given each week after each hemisection operation. All
129 of the BBB score tests, each lasting 5 minutes, were performed in an open field (diameter 150
130 cm) with a wood floor. When we monitored the movement of the hindlimbs, all rats moved
131 freely without any disturbance. The paw placement, joint movements, weight bearing, and
132 coordination among the limbs were used to evaluate the BBB locomotion scale.
133 **2.5 Electrophysiological examinations：**

Before beginning electrophysiological examinations each rat was anesthetized. An electrophysiological examination was given before and after each hemisection operation.

2.5.1 Motor-evoked potential (MEP) studies on the body surface

MEP examination and analyses were performed mainly according to previously published methods (Shen et al. 2016; Yin et al. 2015; Ziegler et al. 2011). Before the examination, the rat was in a relaxed state. Intramuscular electrode needles were implanted in the anterior tibial muscle (TA) and little toe abductor muscle (LTA) on both sides. There were 5 wires used for MEP examination, and each wire was connected to a stainless steel pin. The rostral-caudal locations of the wires were as follows: the first needle was in the midline 5 mm from the nose; the second needle was located subcutaneously in the midline of the head; the third needle was located subcutaneously in the mid-belly; the fourth needle was located in the middle of the muscle being tests; and the fifth needle was located in the tail 3 cm from the root. Stimulations of 10 mA at 1 Hz were administered once per time point: for 1 ms per stimulus. Each muscle received 3 standard stimuli, and the interval time was 30 s.

2.5.2 MEP on the spinal cord

The purpose of the test in spinal cord was to observe the change of conduction from T7-T8 after the operation. Eelectrophysiological examinations involved stimulating microelectrodes and recording microelectrodes (Fig. 3F) (Arvanian et al. 2009; Schnell et al. 2011). The responses evoked by stimulating the ventral horn from the rostral end of T7 to the caudal end of T8 on the ipsilateral side were recorded on the same side or on the contralateral side. The stimulation electrode was positioned approximately 0.7 mm from midline, with a depth of 1.3 mm, and an angle of 25-30° from the vertical sagittal plane. The recording electrodes were positioned approximately 0.7 mm from middle line, with a depth of 1.3 mm and an angle of 15-20° from the

157 vertical sagittal plane. We used the average of two recordings for each side. There was a 30 s
158 interval between the two stimuli. The ventral horn stimulus had a duration of 0.01 ms and a
159 current of 0.5 mA and was delivered at 1 Hz.
160
161 **2.6 Criteria for excluding animals**
162 Rats were excluded from the research according to the following criteria: (1) death during or
163 after the operation; (2) signs of autophagia and/or a serious skin infection; (3) an edematous
164 hindlimb that would affect the BBB score test; (4) death during the electrophysiological
165 recordings.
166
167 **2.7 Statistics**
168 The statistical analysis was performed using SPSS database (version 19.0; SPSS Inc., Chicago,
169 IL, USA). The BBB scores and electrophysiological examination data are shown as means ±
170 SEM. When the data agreed with the Bartley Ball Test, the repeated measures general linear
171 model test was used to determine the overall differences in the different test times after the
172 operation (1 week to 6, 8, or 16 weeks), followed by LSD (least significant difference) tests to
173 make comparisons among groups. P values less than 0.05 were considered statistically
174 significant.
175
176 **3 Results**
177 **3.1 Part 1: Determination of an ipsilateral versus contralateral course**
178 **3.1.1 BBB score s after the first hemisection operation at T7**
179 After the first operation, none of the rats could move their left hindlimb or perform weight-

bearing movements, while the right hindlimb could move slightly. The most significant and rapid improvements in BBB scores of both hindlimbs occurred over the first 2 weeks. BBB scores continued to increase through the 3rd week after the operation and then reached a plateau phase in the 4th week that persisted through the end of the evaluation period at week 8 (Fig. 4A).

3.1.2 BBB scores after a second hemisection operation at T7

A second hemisection at T7 had little effect on movement of either hindlimb in the ipsilateral group. In 7 of 8 rats, BBB scores recovered to the pre-second operation level on the 3rd day and the BBB scores of all rats recovered to the pre-second operation level by the end of the first week following the second operation (Fig. 4D). No significant differences in the BBB scores between the ipsilateral group and the control group were observed after the operation ($p>0.05$, Fig. 4C and D).

After the second hemisection at T7 on the contralateral side, movement in both hindlimbs was instantly obstructed and then started to recover 2 weeks later. By the 4th week, recovery entered a plateau phase. Compared to the rats in transection group, there were no significant differences in BBB scores of the ipsilateral group after the operation ($p>0.05$, Fig. 4E and F). However, there were significant differences in BBB scores between the ipsilateral group and the contralateral group ($p<0.05$, Fig. 4D and E).

3.1.3 Electrophysiological examinations in part 1 of the study

After the second hemisection at T7, the latency periods in both TA muscles and both LTA muscles between rats in the ipsilateral group and control group were not significantly different ($p>0.05$, Fig. 5A-D). However, they were longer in the contralateral group than in the control group ($p<0.05$). There were no significant differences between the contralateral group and the transection group ($p>0.05$). All experimental groups had significantly longer latency periods than

the sham group ($p<0.05$).

There were not significant differences in latency periods in the spinal cord between the sham, control, and ipsilateral groups ($p>0.05$, Fig. 6A and B, and Fig. 3A-C). However, the latency period and wave amplitude disappeared in the contralateral and transection groups after the second hemisection ($p>0.05$, Fig. 6A and B, and Fig. 3D1, D2, E1, E2).

3.2 Part 2: Determination of the involvement of vertebrae T8-T12

3.2.1 BBB scores after a second hemisection operation at T8-T12

In the T8 second hemisection group, BBB scores for the left hindlimb decreased slightly, while BBB scores of the right hindlimb were barely affected. Approximately 3 weeks later, BBB scores of both hindlimbs recovered to the level before the operation ($p>0.05$， Fig. 7A1). In comparisons of BBB scores 6 weeks after the second hemisection, the left hindlimbs in T8-T12 sub-groups displayed poorer and poorer improvement ($p<0.05$， Fig. 7A1-A5). Seven of 8 rats in T12 operation sub-groups exhibited no left hindlimb movement 6 weeks after the operation.

Compared to the operation group, BBB scores for both hindlimbs in the T8-T12 sub-groups in the control group decreased significantly after the second hemisection ($p<0.05$, Fig. 7A1-A5, B1-B5).

BBB scores of both hindlimbs in all T8-T11 sub-groups in the sham operation group recovered completely by the second week after the second hemisection ($p>0.05$, Fig. 7 C1-C4). Compared to the T12 sub-group, the T8-T11 sub-groups displayed better improvement ($p<0.05$， Fig. 7 C1-C5).

3.2.2 Electrophysiological examinations in part 2 of the study

After the second hemisection in the operation group, the latency period in left TA muscles and

left LTA muscles became progressively longer from the T8 sub-group to T12 sub-group ($p<0.05$, Fig. 8A, C).

4 Discussion

4.1 Effects of a second hemisection operation at T7

Many previous animal experiments have shown that after a hemisection operation of the spinal cord, the transplantation of stem cells or various engineered tissue materials in the injury region can improve movement of both hindlimbs. Immunohistochemical examinations have also shown that the nerve fibers in regions rostral to the injury site increased and deeply innervated the lesion site. This indicates that nerves fibers penetrating into the area of injury probably play important roles in recovery after spinal cord injury (Estrada et al. 2014; Fan et al. 2010; Jee et al. 2012; McCall et al. 2012; Wright et al. 2011).

However, some studies using animal models have reached conflicting conclusions. An important example is the work of Courtine et al (Courtine et al. 2008), in which they carried out two successive lesions of the rat spinal cord and showed that the contralateral but not ipsilateral side was essential for recovery.

However, Courtine et al did not performed a second hemisection at the injury region, so whether nerve regeneration within the injury region was attributable to hindlimb movements recovery could not be ruled out (Courtine et al. 2008). Accordingly, in this study, we added an additional experimental group that received a second hemisection in the region of injury.

BBB scores for both hindlimbs decreased significantly after the first hemisection at T7, and 4 weeks later, the improvement reached a plateau. When the second hemisection operation was performed on the injury region, it had almost no effect on movement of either hindlimb ($p>0.05$,

Fig. 4C and D, Fig. 3B1, B2, C1, C2). The MEP results from body surface and spinal cord also displayed no significant differences ($p>0.05$, Fig. 5A-D, Fig. 6A and B, Fig. 3C1, C2, D1, D2). However, in the contralateral and transection groups, hindlimb movement on both sides disappeared after the second operation and there were no significant difference between groups at 8 weeks post-injury ($p>0.05$, Fig. 4E and F). Moreover, MEP in the spinal cord disappeared after the second operation in both groups (Fig. 6A, B and Fig. 3D1, D2, E1, E2). By contrast, we found that, after the spinal cord transection operation MEP activities were still observed at the body surface. For this reason, MEP examination of the spinal cord is a more accurate indicator of injury and recovery than MEP at the body surface.

We thus conclude that the nerve repair in the injury region has no effect after the hemisection operation at T7 on the ipsilateral side, and reparative responses involved nerve fibers on the contralateral side.

4.2 Effects of a second hemisection operation at T8-T12 on the ipsilateral side

4.2.1 T8 subgroup

In T8 subgroup in operation group, the BBB scores of the left hindlimb decreased slightly, from the third week on, all rats returned to the level exhibited before the second operation (Fig. 7A1). Compared to the control group, there were significant differences in the BBB score of both hindlimbs ($p<0.05$, Fig. 7A1, B1). And in the sham group, from the second week on, the sham operation almost had no effect to the movements of both hindlimbs. These results showed that the thoracic vertebra below and next to the spinal cord injury likely had a little assist in the recovery process, which may be affected by neuronal apoptosis around the injury site.

4.2.2 T9 subgroup

In T9 subgroup in operation group, the BBB scores of both hindlimbs decreased slightly and were approximately 1–2 points less than the level before the second hemisection in the 6th week (Fig. 7A2). But the reduction of BBB scores was a little more than in the sham group ($p<0.05$, Fig. 7A2, C2) and were less than in the control group ($p<0.05$, Fig. 7A2, B2). These results showed that the spinal cord at thoracic vertebra T9 likely assisted with the recovery process to some extent.

4.2.3 T10 subgroup

In T10 subgroup in operation group, the BBB scores of left hindlimbs disappeared instantly and began at the 1st week (Fig. 7A3). When compared to the T9 subgroup within the operation group, in the 6th week, the left hindlimb recovered were worse (Fig. 7A2, A3). This finding showed that the spinal cord at thoracic vertebra T10 played an important role in the spontaneous recovery of the spinal cord injury, and the function could not be completely compensated for by other segments of the spinal cord in 6 weeks.

4.2.4 T11 subgroup

In T11 subgroup in operation group, the BBB scores of left hindlimb disappeared instantly and began at the 2ed week (Fig. 7A4). When compared to the T10 subgroup within the operation group, the left hindlimb recovered were worse and the difference was significantly in 6 weeks (Fig. 7A3, A4). This finding showed that the spinal cord at thoracic vertebra T11 played a very important role in the spontaneous recovery of the spinal cord injury.

4.2.5 T12 subgroup

In T12 subgroup in operation group, the BBB scores of left hindlimb disappeared instantly and were approximately 0–1 points from the 2ed to 6th week (Fig. 7A5). These results showed that the spinal cord at thoracic vertebra T12 played a major role in the spontaneous recovery of the spinal

295 cord injury.

296 In summary, results of hemisection at a single vertebra from T8-T12 after an initial
297 hemisection at T7 impaired hindlimb movement recovery in all instances, with the most
298 pronounced effects occurring at T11-T12. Therefore, we conclude based on the data in part 2 of
299 this study that, when caudal to the injury region area, spinal cord segments underlying the T8-
300 T12 vertebrae on the ipsilateral side all participated in the spontaneous recovery process after a
301 hemisection operation at T7. In this case, the T12 vertebral area appeared to be the most
302 important for nerve repair.

303

304 **4.3 Repair processes occurring rostral to the lesion**

305 Many findings have shown that, although the direct conduction pathway was destroyed in rats
306 with a spinal cord injury, commands from brain conducted by the corticospinal tract (CST) can
307 still be transmitted to the lumbar spinal cord below the lesion on the ipsilateral side (Bareyre et al.
308 2004; Courtine et al. 2008; Jankowska & Edgley 2006; Kerschensteiner et al. 2004; van den
309 Brand et al. 2012). After injury, an important mechanism of the spontaneous recovery process is
310 thus that the structure and course of nerve fibers in the CST are remodeled such that they make
311 contact with propriospinal neurons that form detour pathways bypassing the lesion (Nishimura &
312 Isa 2012; Pierrot-Deseilligny 2002; Rosenzweig et al. 2010; Zaaimi et al. 2012). However, the
313 CST is not the only descending tract that affects movement and is not be the only projection
314 system that conveys functional recovery (Han et al. 2013; Hurd et al. 2013). Spared
315 reticulospinal fibers play an important role in the recovery process through spontaneous
316 compensatory sprouting and increases in density after injury; they may also operate caudal to the
317 lesion by enhancing indirect access to reticulospinal commands (Ballermann & Fouad 2006;

318 Zorner et al. 2014). Therefore, we hypothesize that the nerve fiber circuit underlying repair in
319 this study was composed of CST and reticulospinal fibers and propriospinal neurons. We
320 speculate that around the T12 vertebra, which was the most important for nerve repair, there
321 were more nerve fibers relative to the other vertebrae that crossed the midline from the
322 contralateral side to the injury side. However, more research, particularly nerve fiber tracing
323 experiments, is needed to confirm this.
324

325 **4.4 Repair processes occurring caudal to the lesion**

326 Tillakaratne et al. showed that rats exhibited spontaneous recovery via a step-wise process after a
327 complete transection of the mid-thoracic spinal cord, even though the region caudal to the spinal
328 cord lesion did not make any connections to the brain and in absence of descending tracts
329 passing through the lesion (Tillakaratne et al. 2010). Thus, roles of activity of a locally acting
330 central pattern generator (CPG), which is present in many species, are important to consider
331 (Deliagina et al. 1999; Ekeberg & Pearson 2005; Grillner 1985). While, our research argues for
332 the presence of significant spontaneous locomotor recovery resulting from new forms of
333 dynamic control in the spinal CPG from newly generated or remodeled descending tracts, the
334 local CPG still may play the primary role in recovery following spinal cord injury (Rossignol et
335 al. 2007).

336 The CPG of the spinal cord is located in the lumbar enlargement at about the T10-12
337 segments (Magnuson et al. 1999). The second part of this study showed that areas closer to T12
338 vertebra are more important for nerve repair. Therefore, we could conceivably use various
339 treatments to reinforce the role of CPG and the nerve fibers that connect to it to promote
340 recovery. However, given that a second hemisection operation at T9 on the ipsilateral side could

also impair hindlimb movements, which could not recover to pre-operation levels, the CPG is likely not the only factor playing a role in the recovery process.

4.5 Study Limitations

These studies assessed spontaneous recovery from spinal cord injury, but not the impact of any treatment. Further research is needed to confirm if similar results are observed in the case of using a therapeutic intervention within the same experimental injury paradigm; potential therapies include targeting neuroinflammation, transplantation of engineered tissue materials, stem cells, neurotrophic factors, or genetic modifications. We also did not directly assess the path of newly projected nerve fibers to more conclusively and precisely define their course.

5 Conclusions

Our study demonstrates an anatomical mechanism for spontaneous repair processes caudal to spinal cord injury sites in which regenerative fibers cross to the contralateral side, course around the lesion, and then re-cross the midline innervating all caudal, ipsilateral vertebrae with T12 being most important. If we inject stem cells, neurotrophic factors, drugs in the spinal cord around the injury region more than only in the injury region, it might had more effect in the recovery process. Further studies should investigate therapeutic approaches that enhance this process and identify the molecular mechanisms that control it.

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455

7 Figure Legends

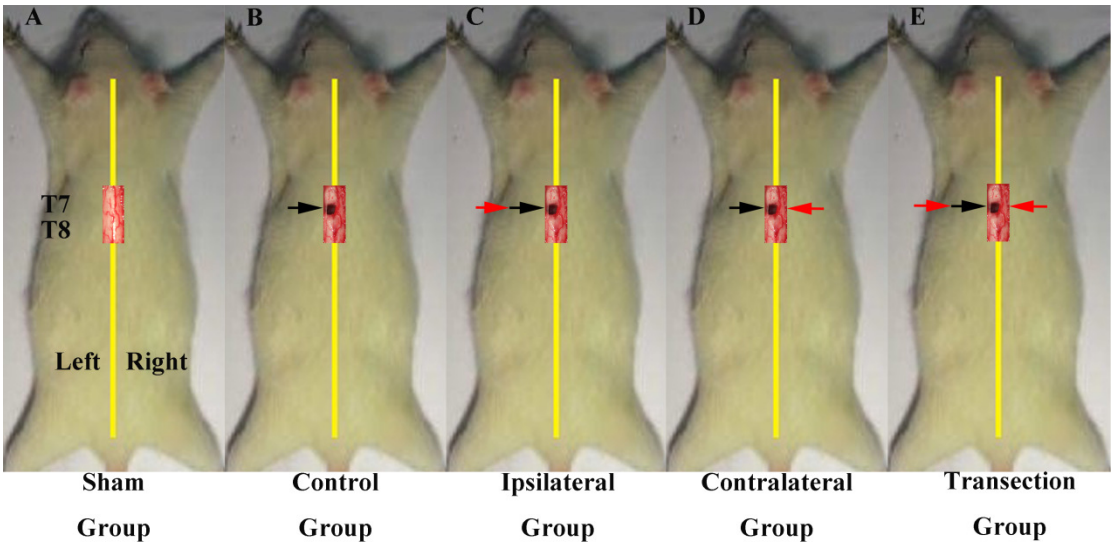
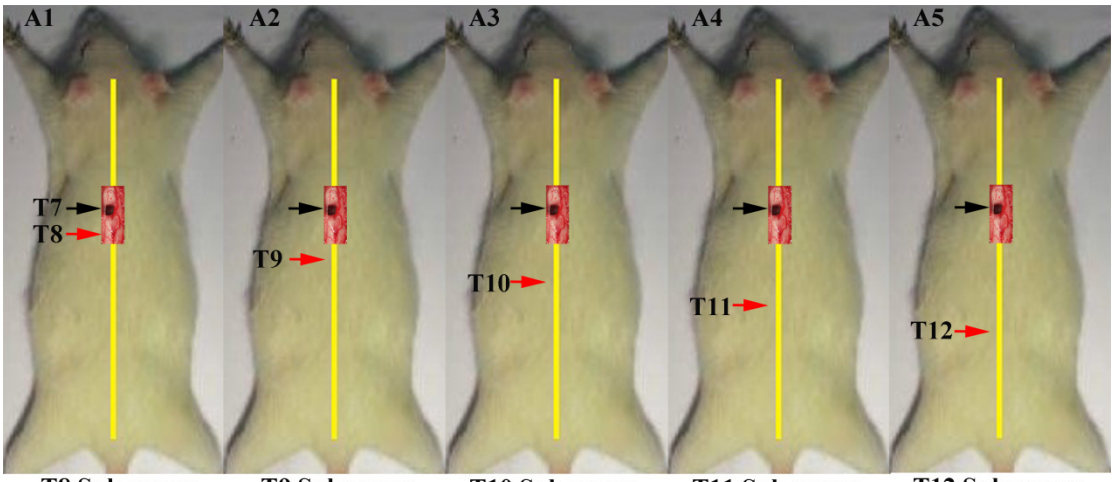
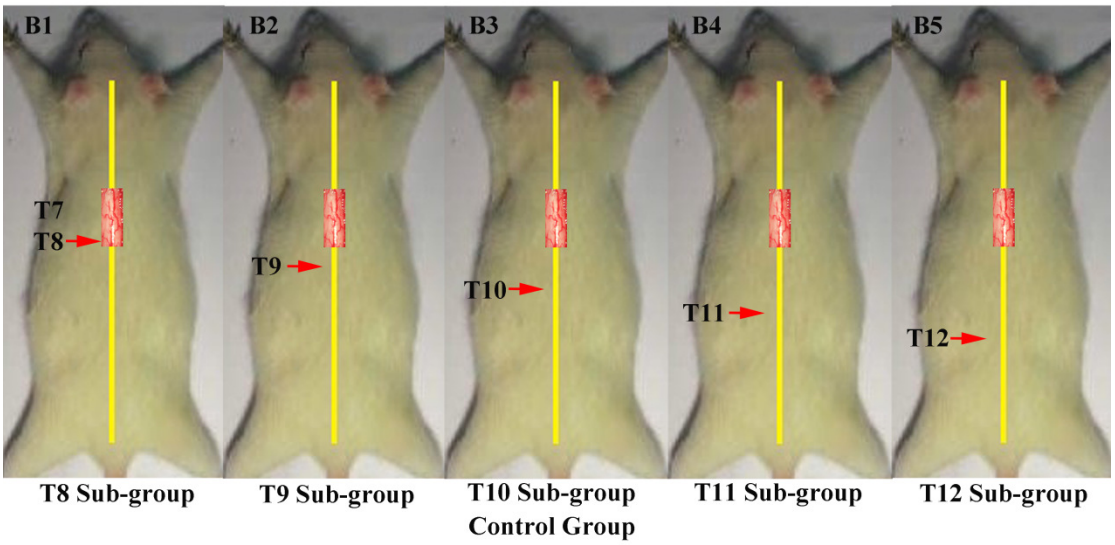


Fig 1. Operations in first part of this study. All the operations were made at T7. A black arrow and a red arrow indicates the level of the first and second hemisection operation separately. (A) The sham group. (B) The control group. (C) The ipsilateral group. (D) The contralateral group. (E) The transection group.


A1
A2
A3
A4
A5
T7
T8
T9
T10
T11
T12
T8 Sub-group
T9 Sub-group
T10 Sub-group
T11 Sub-group
T12 Sub-group
Operation Group


B1
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T7
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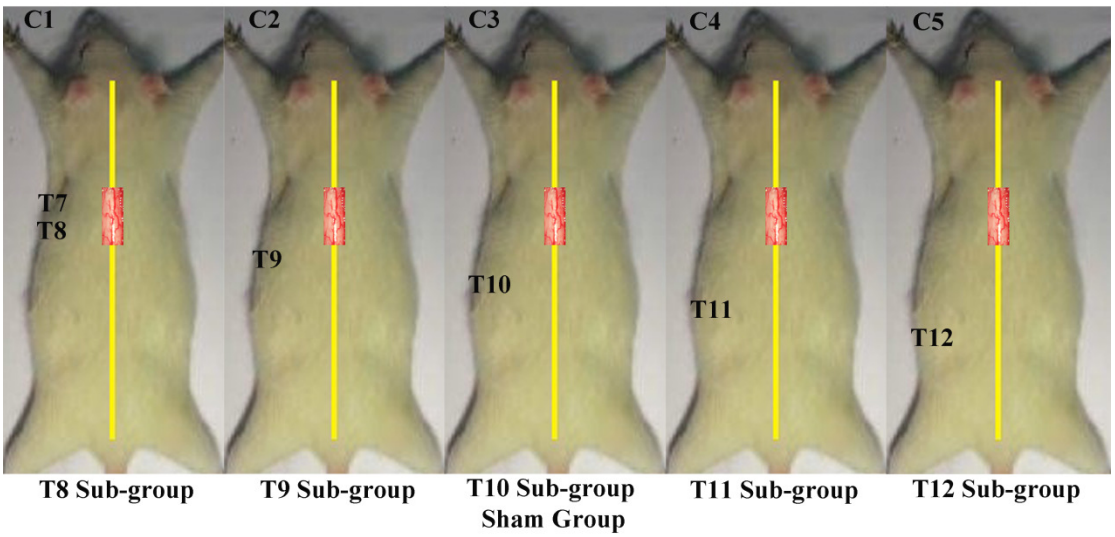
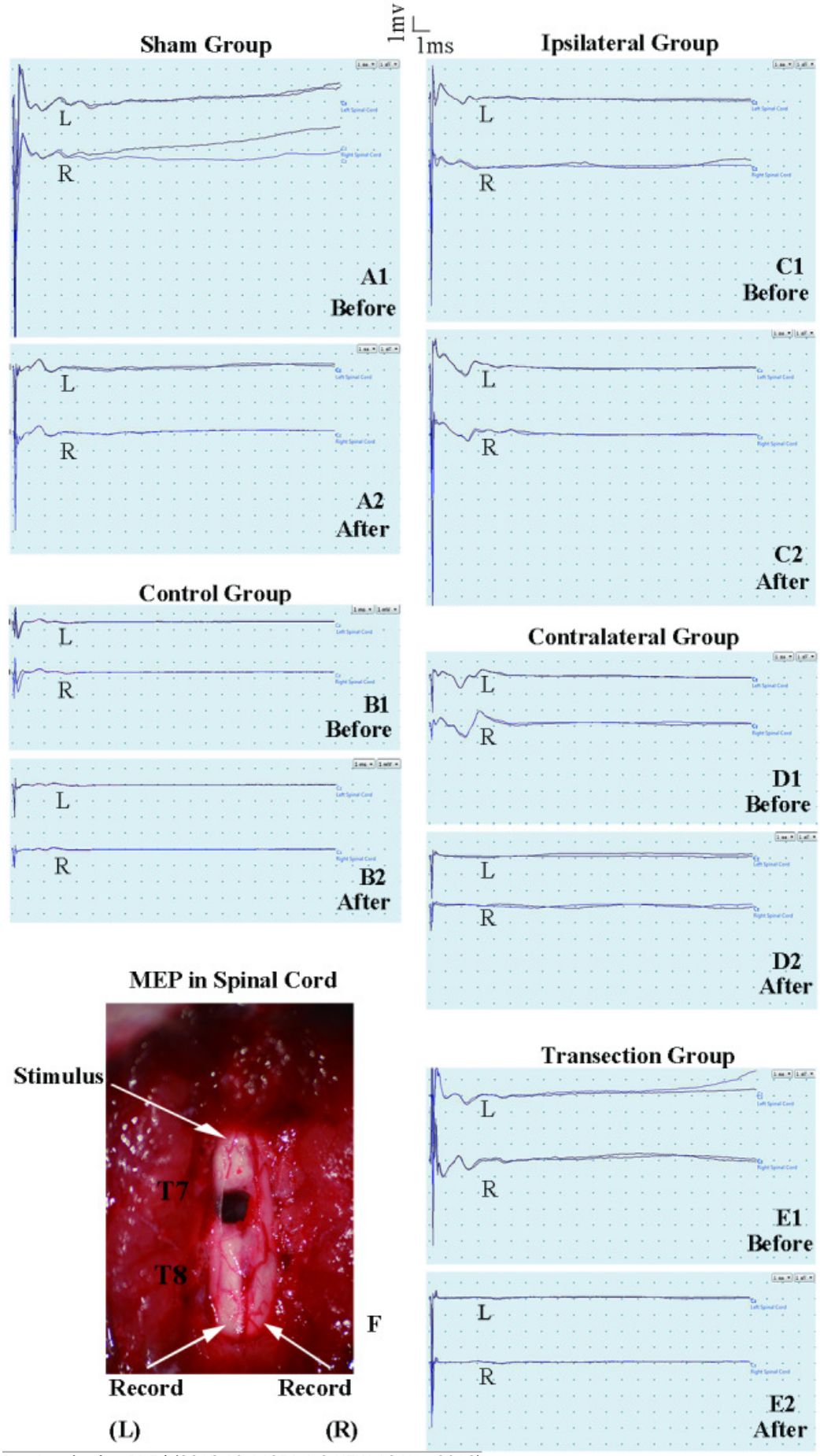
T9 Sub-group
T10 Sub-group
T11 Sub-group
T12 Sub-group
Control Group


C1
C2
C3
C4
C5
T7
T8
T9
T10
T11
T12
T8 Sub-group
T9 Sub-group
T10 Sub-group
T11 Sub-group
T12 Sub-group
Sham Group

467 **Fig 2.** Operations in second part of this study. A black arrow indicates the level of the first
468 hemisection operation; a red arrow indicates the level of the second hemisection operation. (A1-
469 A5) T8-T12 sub-groups in the operation group. (B1-B5) T8-T12 sub-groups in the control group.
470 (C1-C5) T8-T12 sub-groups in the sham group.
471


1mv
1ms
Sham Group
L
R
A1
Before
L
R
A2
After
Control Group
L
R
B1
Before
L
R
B2
After
MEP in Spinal Cord
Stimulus
T7
T8
Record
Record
(L)
(R)
F
Ipsilateral Group
L
R
C1
Before
L
R
C2
After
Contralateral Group
L
R
D1
Before
L
R
D2
After
Transection Group
L
R
E1
Before
L
R
E2
After

473 **Fig 3**. Electrophysiological examinations of the spinal cord in first part of this study before and
474 after the second hemisection operation at T7: (A) Sham group, (B) Control group, (C) Ipsilateral
475 group, (D) Contralateral group, (E) Transection group.

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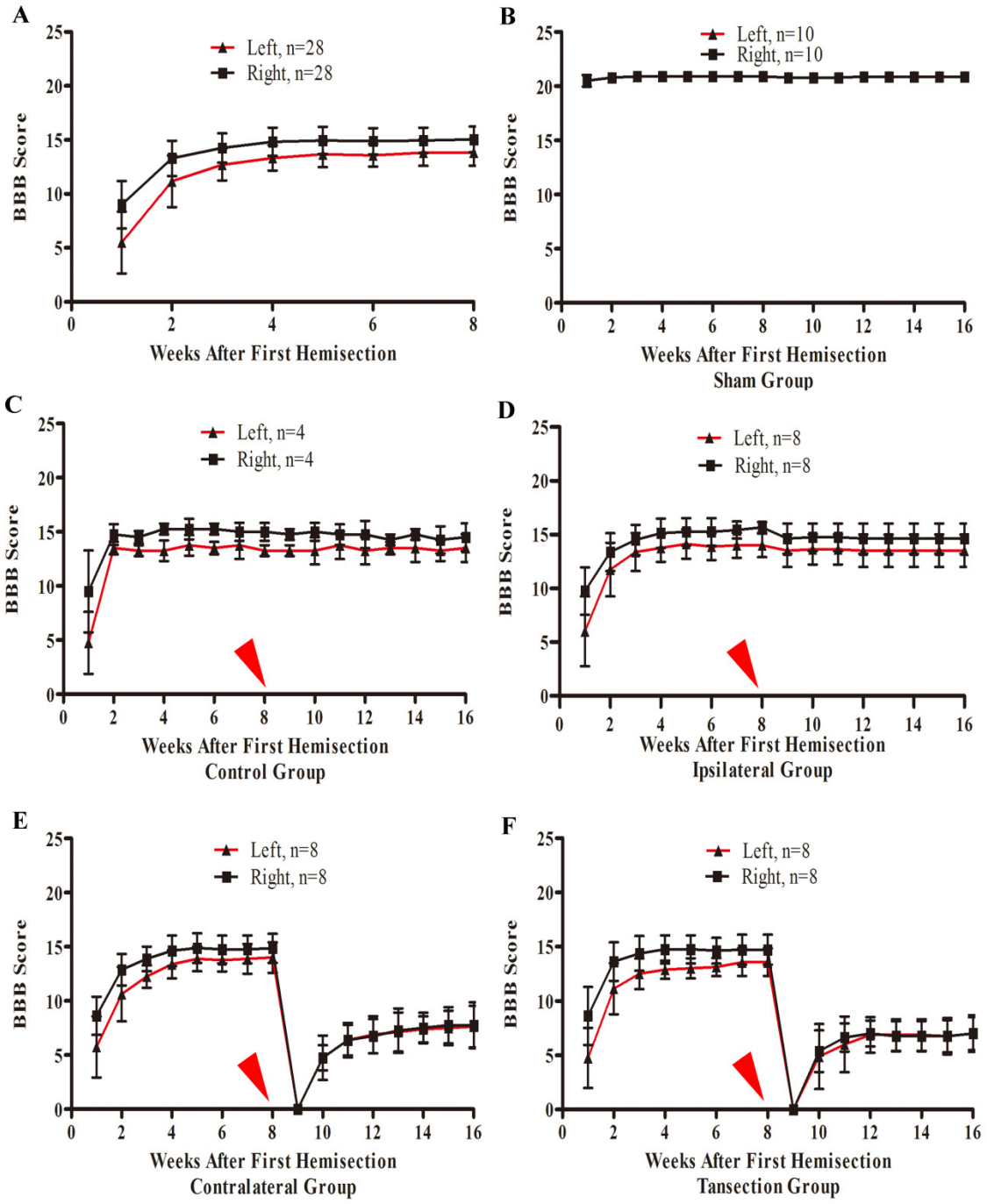


Fig 4. The BBB scores for part 1 of the study. A red arrow indicates the time point for the second hemisection. (**A**) The trend in BBB scores of all rats before the second hemisection operation. (B-E) Trends in BBB scores:(B) Sham group, (C) Control group, (D) Ipsilateral group, (E) Contralateral group, (F) Transection group. Data are presented as mean ± SEM.

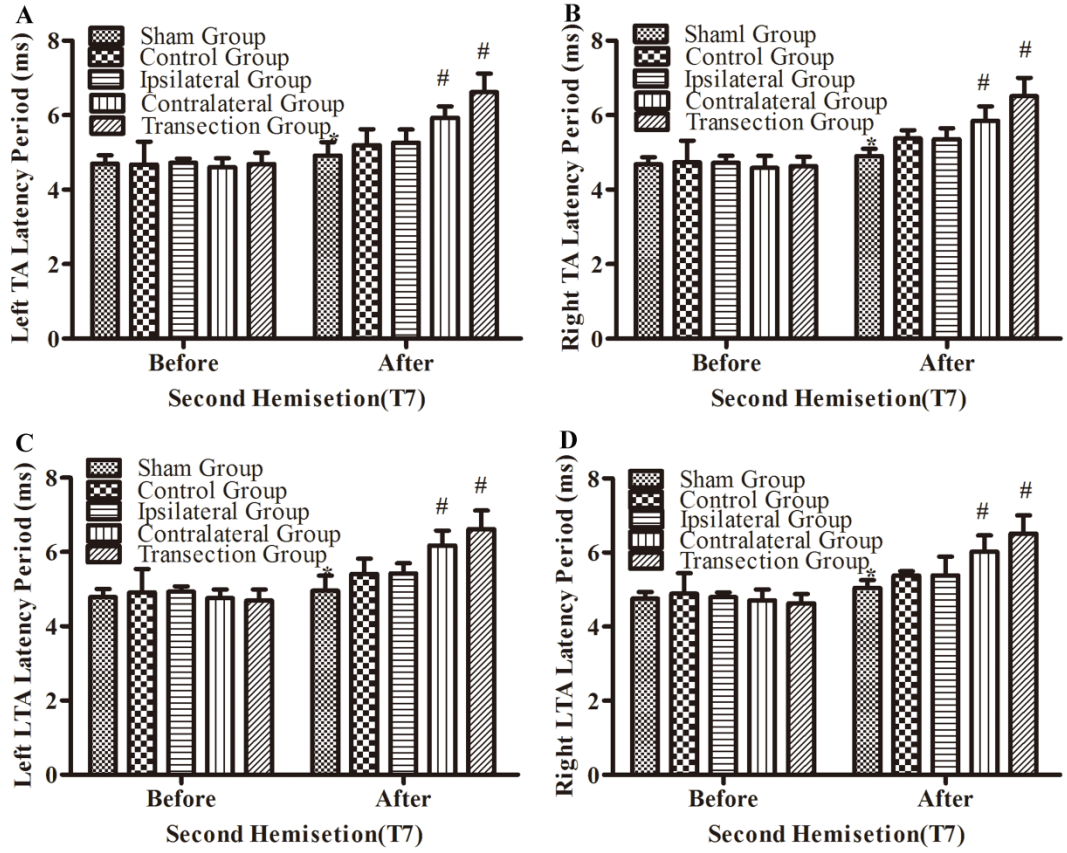


Fig 5. Electrophysiological examinations (MEP, latency periods) in body surface in part 1 part of this study. (A-D) The latency periods in both TA muscles and both LTA muscles. *, P<0.05, compared to the control group. #, P<0.05, compared to the other 4 groups. Data are presented as mean ± SEM.

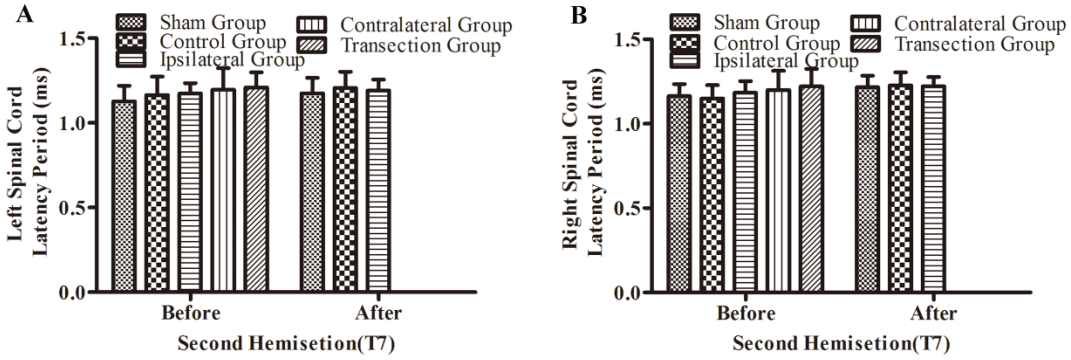


Fig 6.

Electrophysiological examinations (MEP, latency periods) of the spinal cord in part 1 of this study. (A-B) The latency periods on both sides of the spinal cord before and after the second hemisection operation. Data are presented as mean ± SEM.

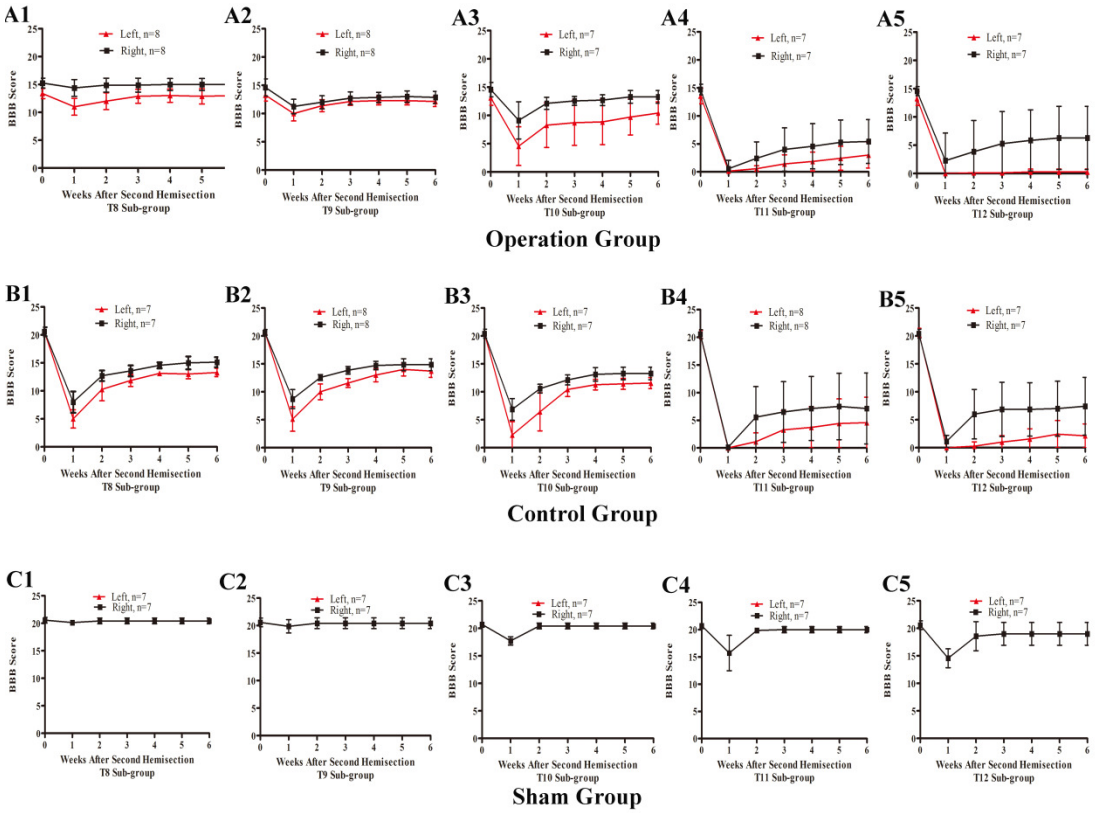
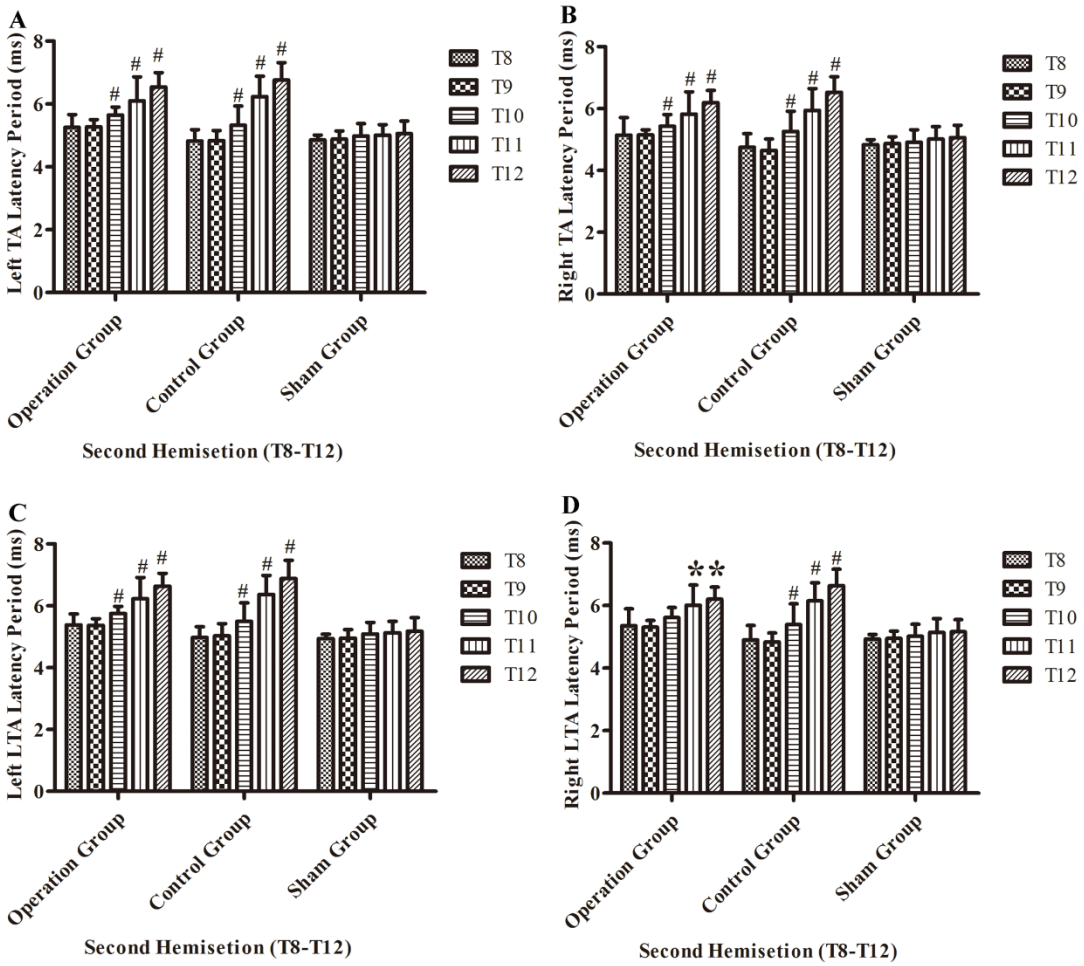


Fig 7. Trends in BBB scores tendency for the T8 to T12 sub-groups in part 2 of the study: (A1-A5) Operation group, (B1-B5) Control group, (C1-C5) Sham group. Data are presented as mean ± SEM.



504

505 **Fig 8**. Electrophysiological examinations (MEP, latency periods) in part 2 of this study after the

506 second hemisection operation at the (A) left TA muscle, (B) right TA muscle, (C) left LTA

507 muscle, (D) right LTA muscle. *, P<0.05, compared to T8 sub-group in each main group. #,

508 P<0.05, compared to the other 4 sub-groups. Data are presented as mean ± SEM.

509

510