The Morphological Evidence Of Spinal Plasticity And Acupuncture In Mammal
Liangfang Wu, Tianran Bao, Deyang Liao
Research Unit of Histology, West China University of Medical Sciences, Changdu 610041, PRC

The plastic ability of the central nervous system (CNS) in adult mammals after traumatic, physiological and age changes is a well known fact now, since the first paper reported by Liu and Chambers (1958) on the plasticity of cat spinal cord under light microscope. Collateral sprouting of axons in intact systems and synaptic reinnervation with target are the main morphological appearances of plasticity in the CNS. There are two types of sprouting: homotypic sprouting and heterotypic sprouting. Homotypic sprouting refers to that the sprouting axons are the same as the injured or influenced system, heterotypic sprouting refers to that the sprouting axons are different from the injured one. Because the spinal cord is the easiest injured part of the CNS and acupuncture of traditional Chinese medicine is one of the major effective method in the treatment of spinal cord hemiplegia and paralysis (Gao 1984, 1985; Li et al 1983, 1985; Luo 1976; Ledergerber 1984). Our laboratory has undergone a series of work, both in vivo and in vitro, on the regularities of collateral sprouting and synaptic reinnervation in spinal lamina II and Clarke's nucleus of the hind limb dorsal rhizotomized cat and the effect of acupoint needling.

The effect of dorsal rhizotomy on the population of synaptic terminals in spinal lamina II

A. The complete dorsal rhizotomy preparation

The number of dorsal root terminals, known as the complex terminals (Wu et al 1986a,b.) which are large terminals making more than one synaptic contacts with postsynaptic profiles (Fig. 1), decreased vigorously and the population of nondorsal root terminals, called the simple terminals (Wu et al 1986a,b.) which are small terminals making one synaptic contact with postsynaptic profile (Fig. 2), increased greatly in spinal lamina II of dorsal horn following hind limb complete dorsal rhizotomy in cats (sectioning L1 to S2 dorsal roots or dorsal root ganglia) (Wang et al un-published). In detail, there remained 11% of the complex terminals in the middle spinal segment (L6) of lamina II, as
compared with normal, but the population of the simple terminals raised to 141% of normal (table 1). This result indicates that heterotypic sprouting is active in complete dorsal rhizotomy preparation. We revealed the presence of an unusual type of synaptic terminals normally not found in lamina II (Wu et al 1986b; Murray et al 1987; Wu & Wang 1990; Chen & Wu 1992). Its morphology was different from complex terminal or and simple terminal. In other words, the size of this newly appeared terminal is as small as the simple terminals, but it always making two small synaptic contacts with two postsynaptic profiles (Fig. 3). It is a type of bisynaptic terminal and we call it the atypical complex terminal and may represent another form of lesion induced plasticity arising from the simple terminals.

Table 1. The percentage of synaptic terminal, as compared to normal, in lamina II of various groups of cat

<table>
<thead>
<tr>
<th>Group</th>
<th>Complex Terminal</th>
<th>Simple Terminal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>24% (L1 19%, L6 11%, S2 41%)</td>
<td>133% (L1 125%, L6 141%, S2 132%)</td>
</tr>
<tr>
<td>2.</td>
<td>51% (L5)</td>
<td>118% (L5)**</td>
</tr>
<tr>
<td>3.</td>
<td>88% (L5)*</td>
<td>124% (L5)**</td>
</tr>
</tbody>
</table>

1. complete rhizotomy (Wang et al, unpublished), 2. partial rhizotomy (Dong et al 1992, 1994), 3. needle partial rhizotomy (Dong et al, 1992, 1994; Dong & Wu 1992), * no significant difference with normal, ** no significant difference between them.

B. The partial dorsal rhizotomy preparation

In lamina II, the population of the complex terminals was higher and that of the simple terminals was lower than that of complete dorsal rhizotomy cat, following partial dorsal rhizotomy (spared L6) (Dong et al, 1992, 1994). As in the middle segment L5, there was 51% of the complex terminals of normal, but the simple terminals was 118% of normal (Table 1). This indicates that the nerve fibers of L6 spared root may undergo sprouting and reinnervating the targets of lamina II - homotypic sprouting. At the same time, the activity of nondorsal root sprouting is somewhat depressed.
The effect of dorsal rhizotomy on the population of synaptic terminals in the Clarke's nucleus

A. The complete dorsal rhizotomy preparation

In the Clarke's nucleus, the number of dorsal root terminals, known as the giant axonal terminals (Rethelyi, 1970; Saito, 1974) - a huge synaptic terminal climbing along the target dendrite and making synaptic active zones with it (Fig. 4) - decreased and the number of one type of nondorsal root terminal, named the small bouton typed terminals (Fig. 4) increased greatly, but the number of another type of nondorsal root terminal, the flatted vesicle terminals (Fig. 5) showed no significant difference as compared with the normal, following hind limb complete dorsal rhizotomy (Zhou et al, 1993). As in the caudal segment of the Clarke's nucleus (L3 level), the number of the giant axonal terminals remained 50% of normal and that of the small bouton typed terminals was 209% of normal (Table 2) We suggest that the remained 50% of the giant axonal terminals may come from the dorsal root caudal to S2 and rostral to L1. This result is similar to those of lamina II, as the heterotypic sprouting is active in complete dorsal rhizotomized cat.

B. The partial dorsal rhizotomy preparation

The population of the giant axonal terminals was higher and that of the small bouton typed terminals was lower than that of complete dorsal rhizotomized cat's Clarke's nucleus (Li and Wu, 1991; Wu et al, 1993a), following partial dorsal rhizotomy, as sparing L6. In detail, in the L3 segment of the Clarke's nucleus, there was 75% of the giant axonal terminals of normal and 177% of the small bouton typed terminals (Table 2). And the number of the flatted vesicle terminals was not significantly different from normal. We suggest that the nerve fibers of spared L6 dorsal root may sprout and reinnervate their targets in the Clarke's nucleus. As this plasticity increases, the activity of heterotypic plasticity decreases too.
Table 2. The percentage of synaptic terminals, as compared to normal, in Clarke’s nucleus (L3) of various groups of cat

<table>
<thead>
<tr>
<th>Group</th>
<th>Giant Axonal Terminal</th>
<th>Small Bouton Typed Terminal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>50%</td>
<td>209%</td>
</tr>
<tr>
<td>2.</td>
<td>75%</td>
<td>177%</td>
</tr>
<tr>
<td>3.</td>
<td>100%</td>
<td>138%</td>
</tr>
</tbody>
</table>

1. complete rhizotomy (Zhou & u 1993); 2. Partial rhizotomy (Li & Wu 1991, Wu et al 1993a); 3. needled partial rhizotomy (Xu et al 1992, Wu et al 1993a); * P<0.05; ** P>0.05

Acupuncture promotes homotypic plasticity of Lamina II and Clarke’s Nucleus in partial dorsal rhizotomy preparation

After partial rhizotomy operation, the cat was needled daily for 20 days. This was divided into two courses. The two pairs of acupoints needled were Zusanli and Xuanzhong, Futu and Sanyinjiao (Fig. 6, Song et al 1992; Inst Acup...1992). These acupoints are the major points which usually are used in treatment of spinal injury and paralysis in traditional Chinese medicine and they are located in the innervating area of L6 spinal nerve also (Nankai Univ 1979).

A. First evidence from bilateral partial rhizotomy

For exploration of the effect of acupuncture, we first used bilateral partial dorsal rhizotomy preparation. As control, one side of spinal cord was operated only. After operation, hind limb on the other side was needled daily for two courses, as the experimental side. After two courses of unilateral acupuncture, the population of the complex terminals in the lamina II of the experimental side was 132% of the control side, but the number of the simple terminals was not significant different from control side (Xiao et al 1989; Wu & Xiao 1992). It indicates that acupuncture may promote the homotypic plasticity in spinal lamina II.

However, the above experiment cannot answer the following questions:
1. Is there any effect of acupuncture on the other side?
2. Is there any influence of acupuncture on the other area of dorsal horn?
For exploration the above questions, we underwent the following experiments on unilateral rhizotomy preparation and divided the animals into rhizotomized group and acupunctured rhizotomized group.

B. In Lamina II

After two courses of acupoints needling, the number of the complex terminals raised to 88% of normal on acupunctured lamina II rhizotomized side. This value was higher than lamina II of the rhizotomized only side (P<0.05) and was not significantly different from normal (P>0.05)(Table 1). The immunoreactive positive area of SP and CCK, which represent the amount of nerve fibers and terminals from dorsal root mainly, increased significantly as compared with the rhizotomized only side and in lamina II of the acupunctured rhizotomized side. But the number of the simple terminals was not significantly different with the rhizotomized side. The number of the complex terminals in unoperated side of both groups was not significantly different. It indicates acupuncture may not influence the opposite side. (Dong et al 1992, 1994, Dong & Wu 1992)

C. In Clarke's Nucleus

The number of the giant axonal terminals in acupunctured rhizotomized side recovered to normal level, but the number of the two types of nondorsal root terminals was not significantly different compared with rhizotomized side Clarke's nucleus (Xu et al 1992, Wu et al 1993a. Table 2). The number of the giant axonal terminals in the unoperated side of both groups was not significantly different too. It means that acupuncture may have no influence on the opposite side.

D. The above result indicates that acupuncture may promote homotypic sprouting of stimulating side, but it has no influence on heterotypic sprouting of the same side's lamina II and Clarke's nucleus. Acupuncture may not influence the contralateral side. We infer that acupuncture may promote homotypic sprouting of dorsal roots in all areas of the dorsal horn of the same side, where dorsal root nerve fibers terminate.
The effect of spared root preparation dorsal horn tissue on the DRG neurite-outgrowth of chick embryo in vitro

A series of experiments in vitro was designed for detection of the effect of lamina II and Clarke's nucleus tissues blocks or tissue extracts from partial dorsal rhizotomized cats on the length of the dorsal root ganglion (DRG) neurite-outgrowth of Hamburger stage 35 chick embryo by modified Maximow double coverslips hanging-drop culture technique (Xue et al 1993c), for preliminary exploration of the mechanism of spinal collateral sprouting.

A. The result from dorsal horn tissue block (explant) cocultured with chick embryo DRG

The length of DRG neurite-outgrowth cocultured with explants from lamina II and Clarke's nucleus of normal of unoperated cat was not significantly different with DRG cultured only (Table 3, 4. P>0.05). It suggests that normal tissue explants may have no special influence on chick embryo DRG neurite. But, the length of DRG neurite-outgrowth cocultured with explants from spared root cat was significantly longer than normal (Fig. 7. Table 3,4. P>0.05) (Wu et al 1993b, Xue et al 1993a). It suggests that partial rhizotomy tissue has neurite-outgrowth promoting activity.

B. The result from various conditioned cultured medium

The length of DRG neurite-outgrowth cultured in a conditioned medium, which added with extract of rhizotomized lamina II of Clarke's nucleus tissue, was longer than that added with extract of normal cat (Fig. 8. Table 3, P<0.05) (Liao et al 1993a, Xue et al 1993b). It suggests that the neurite-outgrowth promoting factors of rhizotomized dorsal horn may be present in extracellular and intracellular fluid.
Table 3. The effect of spared root cat Lamina II tissue on the length of chick embryo DRG neurite-outgrowth (X±SE μm)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Culture</th>
<th>24 hr Length</th>
<th>P Value</th>
<th>48 hr Length</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>19</td>
<td>103.01±2.19</td>
<td>&gt;0.05</td>
<td>196.72±26.29</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>A2</td>
<td>26</td>
<td>120.48±9.39</td>
<td>&lt;0.05</td>
<td>294.14±20.13</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>A3</td>
<td>26</td>
<td>228.70±11.18</td>
<td>&lt;0.05</td>
<td>460.07±32.20</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>B2</td>
<td>31</td>
<td>165.76±29.27</td>
<td>&lt;0.05</td>
<td>208.61±10.00</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>B3</td>
<td>31</td>
<td>214.76±29.27</td>
<td>&lt;0.05</td>
<td>412.61±31.48</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>B3</td>
<td>24</td>
<td>127.45±16.74</td>
<td>&lt;0.05</td>
<td>204.18±86.14</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>B4</td>
<td>27</td>
<td>255.23±8.73</td>
<td>&lt;0.05</td>
<td>427.26±15.32</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

A: tissue block, B: tissue extract, 1: DRG only, 2: unoperated, 3: rhizotomy, 4: needled rhizotomy.
Table 4. The effect of spared root cat Clarke’s Nucleus tissue on the length of ratio of chick embryo DRG neurite-outgrowth (X± SE µm)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Culture</th>
<th>Ratio 24 hr (Length µm)</th>
<th>P Value</th>
<th>Ratio 48 hr (Length µm)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>28</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(89.10±8.76)</td>
<td></td>
<td>(180.10±39.64)</td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>30</td>
<td>1.34±0.15</td>
<td>&lt;0.05</td>
<td>1.30±0.14</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(117.18±14.95)</td>
<td></td>
<td>(229.86±40.45)</td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td>26</td>
<td>2.03±0.24</td>
<td></td>
<td>1.82±0.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(175.25±16.77)</td>
<td></td>
<td>(320.19±44.60)</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>38</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(118.31±9.55)</td>
<td></td>
<td>(278.37±29.11)</td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td>38</td>
<td>1.17±0.10</td>
<td>&lt;0.05</td>
<td>1.11±0.08</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(134.97±4.75)</td>
<td></td>
<td>(309.36±28.02)</td>
<td></td>
</tr>
<tr>
<td>B3</td>
<td>32</td>
<td>1.74±0.12</td>
<td></td>
<td>1.54±0.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(202.29±7.25)</td>
<td></td>
<td>(421.82±26.16)</td>
<td></td>
</tr>
<tr>
<td>B4</td>
<td>22</td>
<td>1.66±0.16</td>
<td>&lt;0.05</td>
<td>1.55±0.15</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(204.14±21.39)</td>
<td></td>
<td>(338.00±43.68)</td>
<td></td>
</tr>
<tr>
<td>B4</td>
<td>22</td>
<td>2.43±0.27</td>
<td></td>
<td>2.11±0.19</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(292.20±21.79)</td>
<td></td>
<td>(470.06±72.08)</td>
<td></td>
</tr>
</tbody>
</table>

A: tissue block, B: tissue extract, 1: DRG only, 2: unoperated, 3: rhizotomy, 4: needled rhizotomy.
The effect of acupunctured rhizotomized tissue extract on DRG neurite-outgrowth of chick embryo in vitro

In this series of experiments, the rhizotomized cat was needled with the same two pairs of acupoints as before, but one course or ten days was applied. The extracts form lamina I or Clarke’s nucleus tissue were added to the culture medium to explore the effect of acupuncture on chicken embryo DRG neurite-outgrowth.

The DRG neurite-outgrowth length of acupuncture spared root preparation lamina II extract was longer than that of spared only group extract (Liao et al. 1993b, Table 3). The neurite-outgrowth length of acupunctured rhizotomized cat Clarke’s nucleus extract was longer than that of rhizotomized only extract too (Xue et al. 1994, Table 4). The result indicates that the neurite-outgrowth promoting activity of acupunctured rhizotomized extract was stronger than that of rhizotomized only extract.

Conclusions

The following conclusions can be obtained:
1. The neurite-outgrowth promoting activity of lamina II and Clarke’s nucleus tissue or extract may be involved in the collateral sprouting of spared root in dorsal horn and this activity is enhanced by acupuncture.
2. The tissue extract of lamina II and Clarke’s nucleus from rhizotomized or acupunctured rhizotomized preparation may contain some diffusible chemical substances, which involve in the neurite-outgrowth promoting activity and collateral sprouting.

References


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Luo FQ (1976) “Electroacupuncture Pathway” technique treatment 68 cases of paralysis. (Chin) New Med 1:20


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Legend

Fig. 1  Electron micrograph of lamina II neuropil, showing complex terminal (CT) with six synaptic contacts.

Fig. 2  Electron micrograph of lamina II neuropil, showing a simple terminal (ST) with one synaptic contact.

Fig. 3  Electron micrograph of lamina II neuropil, showing an atypical complex terminal (ACT) with 2 synaptic contacts.

Fig. 4  Electron micrograph of Clarke's nucleus neuropil; Ωa giant axonal terminal making several synaptic active zones with a dendrite (D); ]+=a small bouton typed terminal.

Fig. 5  Electron micrograph of Clarke's nucleus neuropil. Ωa flattened vesicle terminal.

Fig. 6  Diagram showing location of four acupoints. a. lateral view, b. medial view.

Fig. 7  The photo of DRG(D) cocultured with lamina II explant (E) for 48 hr. a. normal explant, b. rhizotomy explant.

Fig. 8  The photo of neurite-outgrowth of DRG cultured in conditioned medium added extract form normal (a) and rhizotomy (b) Clarke’s nucleus tissue for 48 hr.