Constituent Ratio of Motor Fibers From the C5-C7 Spinal Nerve in the Radial Nerve Is Greater in Pup Rats Than in Adult Rats

MINGBO NIE, MD; LIANG CHEN, MD; YUDONG GU, MD

abstract

Clinically, injuries of C5-C7 of the brachial plexus cause falling of the wrist and fingers in infants but not in adults unless 4 consecutive spinal nerves are injured. The purpose of this study was to compare the constituent difference of spinal nerves in the radial nerve between pup and adult rats.

A group of 16 pup rats and a group of 16 adult rats were each divided into 2 groups of 8 (P1 and A1 groups, C5-C6 were divided; P2 and A2 groups, C5-C7 were divided). A nerve conduction study and histological examination were performed to evaluate radial nerve innervation to the extensor digitorum communis muscle after dividing the spinal nerves. Retrograde tracing with 5% cholera toxin B for anterior horn motoneurons of the spinal cord innervating the radial nerve was performed in 8 pup rats and 8 adult rats. Results showed that the division of C5-C7 caused more significant damage to radial nerve innervation to the extensor digitorum communis in pups than in adults, although the division of C5-C6 did not. In pups, the percentages (median with interquartile) of anterior horn motoneurons of the spinal cord innervating the radial nerve were 36.4 (28.3-38.5) in C5-C6, 28.1 (24.5-32.5) in C7, and 37.5 (36.5-39.3) in C8-T1. In adults, they were 24.2 (23.6-27.8) in C5-C6, 21.8 (19.5-26.3) in C7, and 50.7 (48.7-55.5) C8-T1.

This study implies that C7 innervation in the radial nerve in humans may be more critical to the function of this nerve in infants than in adults.

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Drs Nie, Chen, and Gu have no relevant financial relationships to disclose.

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doi: 10.3928/01477447-20120525-32
Obstetric brachial plexus palsy is a traction lesion of the spinal nerves of the brachial plexus occurring during delivery. Two pathological types exist: Erb’s palsy, the most common type, involves the C5-C6±C7 spinal nerves, and total palsy involves C5-T1. Typical symptoms of C5-C7 Erb’s palsy include the loss of shoulder abduction, elbow flexion, and wrist–finger extension. However, in traumatic brachial plexus injuries in adults, symptoms of C5-C7 palsy are similar to those of C5-C6 palsy, which involve the shoulder and elbow; falling of the wrist and fingers does not occur unless 4 consecutive spinal nerves of the brachial plexus are injured. This is the rationale for transferring the healthy ipsilateral C7 to the avulsed upper trunk for adults with brachial plexopathy. The least number of injured spinal nerve roots that causes falling of the wrist and fingers is higher than that of infants. implies that motor components from spinal nerves in the radial nerve change as infants grow. The authors hypothesized that motor fibers in the radial nerve originated mainly from C5-C7 in infancy, but those from C8-T1 gradually took over until adulthood, after which C8-T1 innervation compensated for the function of the radial nerve after the loss of C5-C7.

The rat has been extensively used as an ideal animal model for the study of brachial plexus injury in humans because its brachial plexus is similar in structure and function to that of a human. In rats, the radial nerve branches out from the posterior cord, which originates from the C5-T1 spinal nerves of the brachial plexus and innervates the triceps and forearm extensors. For the current study, an investigation was designed with a rat model to compare the constituent differences of spinal nerves in the radial nerve between pup and adult rats. Elucidation of regulation for those changes during postnatal development in the rat may help one understand the different clinical appearances of C5-C7 injuries in newborn and adults humans.

Materials and Methods

Sixteen Sprague-Dawley rat pups aged 7 days and 16 Sprague-Dawley adult rats aged older than 2 months weighing 250 to 300 g were enrolled in this study. The 16 rat pups were divided into 2 groups of 8: the P1 group, in which the C5-C6 spinal nerves of the brachial plexus were divided at the right side; and the P2 group, in which C5-C7 were divided. The 16 adult rats were also divided into 2 groups of 8: the A1 group, in which the C5-C6 spinal nerves were divided at the right side; and A2 group, in which C5-C7 were divided.

The rats were anesthetized with 1% sodium pentobarbital (50mg/kg) by intraperitoneal injection and placed in the prone position. By aseptic techniques and a 16× microscope, the brachial plexus was exposed through a posterior middle incision along the neck. The spinal nerves of the brachial plexus at the right side were recognized by the landmark of the T2 spinal process. The C5-C6 or C5-C7 spinal nerves were divided according to grouping, and the proximal and distal ends of the spinal nerves were embedded in the surrounding tissue to prevent reinnervation of the spinal nerves. The left brachial plexus was kept intact as a control. After recovery from anesthesia, adult rats were permitted to move freely, and pup rats were returned to their mothers.

Three months after the division of the spinal nerves, a nerve conduction study was performed to evaluate the radial nerve innervation to the extensor digitorum communis muscle, which is the representative extensor of the forearm innervated by the deep branch of the radial nerve. Under anesthesia, the extensor digitorum communis muscle with the radial nerve was exposed at both sides. Using a 2000M electromyograph (Dantec, Tonsbakke Denmark), the nerve-muscle electric latency and compound muscle action potentials of the motor nerve of the radial nerve with extensor digitorum communis distribution were determined. The values of latency and amplitude of compound muscle action potentials of the radial nerve at the experimental (right) side were expressed as percentages of those at the control (left) side.

After the nerve conduction study, a histological examination was performed of the deep branch of the radial nerve at both sides. Three mm of the deep branch of the radial nerve was harvested, fixed with 4% paraformaldehyde, embedded in paraffin, sliced at 0.5 µm, and stained with toluidine blue. The number of myelinated nerve fibers in the sample nerve was counted at 40× magnification with the FW2000 image analysis system (Leica Microsystems, Wetzlar, Germany). The value of the experimental side was expressed as the percentage of that of the control side.

The extensor digitorum communis muscles at both sides were removed for determination of the cross-sectional muscle fiber area. The specimen was fixed with 4% paraformaldehyde, embedded in paraffin, and cut into 5-µm-thick sections. Hematoxylin-eosin staining was applied to every fifth section, for a total of 5. The average cross-sectional area of the muscle fiber was obtained from measurements of the 5 sections, and the value at the experimental side was expressed as the percentage of that at the control side.

Retrograde Tracing of Anterior Horn Motoneurons of the Spinal Cord Innervating the Radial Nerve

After anesthesia, the right radial nerve in the cubital fossa was exposed and cut. The proximal end of the radial nerve was put in a soft tube filled with 5% chola toxin B (Sigma-Aldrich, St Louis, Missouri) for at least 45 minutes. The surrounding area was completely covered with small pieces of gauze to avoid leakage of the tracer in it. The gauze and soft tube were removed, and the operating field was extensively rinsed with saline before the incision was closed.

After survival of 24 to 48 hours, the rat was reanesthetized and perfused transcardially with 100 mL of 0.1M phosphate buffered 0.9% saline (pH 7.4), followed by 300 mL of 4% paraformaldehyde in phosphate-
buffered saline at 4°C. The spinal cord from C5-T1 was removed with the dorsal root ganglia attached, which was used for the determination of the border between the 2 adjacent spinal segments. The removed spinal cord was placed in the 4% paraformaldehyde fixative for 6 hours and then transferred to 30% sucrose in phosphate-buffered saline at 4°C for 10 to 12 hours overnight. The spinal cord from C5-T1 was divided into 3 blocks: C5-C6, C7, and C8-T1. Each block was sectioned transversely at 30 µm thickness on a freezing microtome. Quantitative analysis was performed on every fifth section of the C5-C6 (n=20), C7 (n=10), and C8-T1 (n=20) segments. The sections were incubated for 10 to 12 hours overnight at 4°C with rabbit anti-cholera toxin B antibody (1:500; abcam, Cambridge, Massachusetts) in phosphate-buffered saline containing 0.3% Triton X-100 (Sigma-Aldrich, St. Louis, Missouri) and 5% normal donkey serum, followed by incubation with Alexa Fluor 594-conjugated donkey anti-rabbit IgG antibody (1:1000; Invitrogen, Eugene, Oregon) for 1 hour to show cholera toxin B–positive cells. Then, the nuclei were counterstained with 4′,6-Diamidino-2-phenylindole (DAPI, 1:1000; Sigma-Aldrich, St Louis, Missouri) for a half hour. Finally, the sections were coverslipped with mounting medium (Vector Laboratories, Burlingame, California).

Images were obtained on an epifluorescent Nikon microscope equipped with a Nikon Coolpix digital camera (Tokyo, Japan) and a Leica TCS SP5 confocal microscope (Mannheim, Germany). All images were imported into Adobe Photoshop version 7.0 software (San Jose, California) and were not manipulated other than slight modifications of the contrast and brightness settings. The image counterstained by DAPI and that stained immunohistochemically by cholera toxin B were merged to obtain a clear and intact image of the anterior horn motoneurons of the spinal cord. The labeled anterior horn motoneurons, which are located in laminae IX 10, were counted, and the diameters of their nuclei were measured at random from 8 to 10 anterior horn motoneurons in 1 section. The number of cholera toxin B–labeled anterior horn motoneurons in C5-C6, C7, and C8-T1 were gained from Abercrombie’s formula,11 which can correct double-count errors (ie, corrected count = primary count × section thickness/average nucleus diameter + section thickness). The percentage of cholera toxin B–labeled anterior horn motoneurons in C5-C6 was calculated by dividing the positive anterior horn motoneurons in C5-C6 by the total positive anterior horn motoneurons from C5-C6, C7, and C8-T1 and multiplying by 100%; those in C7 and C8-T1 were obtained by analogy.

All surgical procedures and protocols used were in accordance with the Guidelines for Ethical Care of Experimental Animals approved by the International Animal Care and Use Committee.

Statistical Analysis
All data were expressed as median with interquartile range (p25-p75). The Mann-Whitney U test was used to compare variables between the pup and adult groups. Statistical data were analyzed with SPSS Statistics version 19 software (IBM, Chicago, Illinois). All tests were 2-tailed, and statistical significance was set at P=.05.

RESULTS
Nerve Conduction Study
After dividing the C5-C6 spinal nerves, percentages (median with interquartile) for latency and those for amplitude of compound muscle action potentials of the radial nerve with extensor digitorum communis muscle distribution at the right side were 116.4 (88.9-138.5) in the P2 group and 115.9 (73.1-149.0) in the A2 group, and no statistical difference existed between the 2 groups (z=–0.000, P=1.000); those for amplitude of compound muscle action potentials were 56.9 (80.6-83.5) in the P2 group and 83.3 (69.1-96.1) in the A2 group, and the difference between the 2 groups was statistically significant (z=–3.361, P=.001).

Myelinated Nerve Fiber Count
After dividing the C5-C6 spinal nerves, percentages for the number of myelinated nerve fibers in the deep branch of the radial nerve at the right were 92.3 (78.8-102.6) in the P1 group and 96.0 (87.1-108.7) in the A1 group, and no statistical difference existed between the groups (z=–1.155, P=.248); after dividing C5-C7, the percentages were 54.1 (40.1-67.3) in the P2 group and 86.3 (72.4-109.4) in the A2 group, and the former was statistically less significant than the latter (z=–3.153, P=.002).

Muscle Histomorphometry
After dividing the C5-C6 spinal nerves, the percentages for the cross-sectional area of the muscle fiber of the extensor digitorum communis muscle were 89.2 (78.2-97.8) in the P1 group and 92.5 (82.4-102.4) in the A1 group, and no statistical difference existed between the groups (z=–1.155, P=.248); after dividing C5-C7, the percentages were 55.2 (49.3-62.3) in the P2 group and 74.7 (65.3-85.1) in the A2 group, and a statistical difference existed between the groups (z=–2.941, P=.003).

Retrograde Tracing of Anterior Horn Motoneurons of the Spinal Cord Innervating the Radial Nerve
After retrograde perfusion with cholera toxin B from the radial nerve, all analyzed cross-sections from the C5-C6, C7, and C8-T1 segments of the spinal cord contained cholera toxin B–labeled anterior horn mo-
toneurons, which were clustered with cholera toxin B staining mainly in the cytoplasm. Although similar in shape, the anterior horn motoneurons of the adult rats appeared to be bigger than those of the pup rats (Figure 1).

In regard to the percentages of anterior horn motoneurons innervating the radial nerve, those in C5-C6 and C7 were statistically higher in the pup group than in the adult one, respectively, whereas percentages of anterior horn motoneurons in C8-T1 were statistically lower in the former than in the latter (Figure 2; Table).

**DISCUSSION**

Falling of the wrist and fingers is a typical symptom of Erb’s palsy. Characteristic pathological appearances include ruptures of the C5-C6 spinal nerves and avulsion of C7, while C8-T1 are intact. However, palsy of the radial nerve does not occur in adult brachial plexopathy with the same spinal nerve lesions. In the authors’ early experience with surgical reconstruction for Erb’s palsy, they repaired the upper trunk but left the avulsed C7 nerve alone. The authors stress the need for simultaneous repair of C7 because it is important for gaining good recovery of extension of the wrist and fingers. Falling of the wrist and fingers occurring in C5-C7 Erb’s palsy in infants but not in that of adults suggests that the constituent ratio of motor fibers from spinal nerves in the radial nerve is different between the 2 age groups.

In the P2 group, in which the C5-C7 spinal nerves of the pup rat were divided, the results of the nerve conduction study, of myelinated nerve fiber count in the deep branch of the radial nerve, and of histomorphometry of the extensor digitorum communis muscle were mostly statistically worse than those of the A2 group, in which the C5-C7 spinal nerves of the adult rats were cut. However, the results of those determining items were not statistically different between the P1 and A1 groups, in which the C5-C6 spinal nerves were divided. These outcomes imply that although impairment was not more significant in the pup rat with C5-C6 palsy, functional damage of the radial nerve is more severe in pup than in adult rats where the C5-C7 spinal nerves are injured. Therefore, this rat model successfully simulates the clinical appearances of falling of the wrist and fingers occurring in Erb’s palsy with C5-C7 lesions but not in adult plexus injury with the same nerve lesions.

Cholera toxin B can bind to the GM1-ganglioside receptor, which is located on the surface of the neuron. Because it is a highly sensitive retrograde neuroanatomic tracer, cholera toxin B has been widely applied in the study of the location of neurons. In the current study, the authors observed and quantitatively analyzed the labeled neurons in the lamina I of the spinal cord in which only motoneurons are located. Results of the current study showed that in regard to motor fibers from spinal nerves in the radial nerve, the contributions of the C5-C6, C7, and C8-T1 segments were significantly different between the pup and the adult groups. In the radial nerve of the pup...
rat, the number of motoneurons of the C5-C7 spinal nerves accounted for nearly two-thirds of that of the C5-T1 spinal nerves and one-third of that of C8-T1, whereas in the adult rat, the number of motoneurons of the C8-T1 segment was equal to or more than that of C5-C7 (Table 4). These outcomes confirm that the constituent ratio of motor fibers from spinal nerves in the radial nerve changes during postnatal development of the rat (ie, the ratio of motor elements from C5-C7 lessens and that from C8-T1 increases).

Some studies have reported that the natural death of neurons or lessenning of axons can occur during normal postnatal development. Korak et al\(^\text{15}\) reported that the significant contribution of C7 to the musculocutaneous nerve motoneuron pool observed after birth was lost during maturation. Chung and Coggeshall\(^\text{16}\) reported a decline of 56% in the axonal number of the neonatal rat dorsal funiculus, with no death of corresponding neurons, from 2 weeks postnatal to adulthood. Huang et al\(^\text{17}\) reported that some sympathetic preganglionic neurons projected to the superior cervical ganglion via the C7 spinal nerve in the pup rat but this pathway disappeared during postnatal development. The implication for these nerve fiber changes during postnatal development, including those of the current study, may lie in the fact that the nervous system needs to be adapted to more subtle function of the target organ during the course of development.

The original characteristics of the spinal nerves for motor fibers of the radial nerve in the pup rat suggest that in an operation for an infant with Erb’s palsy where the C7 spinal nerve is avulsed, the repair of C7 should not be ignored because the contribution of C8-T1 to the radial nerve may not be as much as that in the adult. By the same principle, an operation with ipsilateral C7 transfer to the avulsed upper trunk, which is often done in adult patients with C5-C6 avulsion, might be avoided in infants.

**CONCLUSION**

This study demonstrates that motor fibers from spinal nerves of the brachial plexus in the radial nerve originate mostly from C5-C7 in pup rats, but those from C8-T1 are increased at adulthood when the ratio of motor fibers from C8-T1 approximates or exceeds that from C5-C7. This development change in the radial nerve of the rat implies that C7 innervation may be more critical to the function of this nerve in infants than in adults.

**REFERENCES**


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**Table**

Results of Retrograde Tracing for Anterior Horn Motoneurons of the Spinal Cord Innervating the Radial Nerve in Rats\(^\text{a}\)

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\(^{a}\)Values are expressed as median with interquartile. [AQ 3]

\(^{b}\)N=8.

\(^{c}\)The percentage of labeled anterior horn motoneurons in C5-C6 was calculated by dividing the number of anterior horn motoneurons in C5-C6 by the total number of anterior horn motoneurons in C5-C6, C7, and C8-T1 and multiplying by 100%; those in the C7 and C8-T1 were obtained by analogy.

\(^{d}\)Mann-Whitney U test.

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\[AQ 26-what does count mean?\]


