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NOTE

Anatomy

Innervation of wing membrane in Japanese little horseshoe bats, *Rhinolophus cornutus*

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ABSTRACT. The spinal nerves supplying the wing membranes of Japanese little horseshoe bats, *Rhinolophus cornutus* were studied. The wing membrane was innervated by nerve branches of the radial, ulnar, and median nerves, showing that the membrane was formed from the skin of the forelimb rather than that of the thoracolumbar skin. The radial nerve was mainly composed of the ventral rami of C7–T1, the ulnar nerve by C8–T2, and the median nerve by C8–T1. These components of *R. cornutus* tended to be from a narrower range of spinal nerves and to position more caudally than those of humans. In addition, the ulnar nerve showed a distribution pattern different from that of other mammals.

KEY WORDS: components of spinal nerve, median nerve, radial nerve, ulnar nerve, wing membrane

The surface area of the forelimbs of bats is much larger than that of other mammals as they have a wing membrane. This is considered to be one of the most sensitive regions, because of the presence of many mechanoreceptors controlling the cutaneous sensation which seems to be related to flight [12]. However, the components of the dominant nerve of the wing membrane are still unclear. Although a recent study using neuron tracking reported the components of the nerves to the wing membrane [8], and showed the rough distribution of nerves in each region of the wing, the details are still not well defined. In this study, we investigated the nerves that supply the wing membrane of Japanese little horseshoe bats, *Rhinolophus cornutus,* family Rhinolophidae. This is an indigenous species to Japan and inhabits the entire area of the Japanese archipelago except the Yaeyama Islands [7].

Three wild Japanese little horseshoe bats (2 males and 1 female) collected from Nasukarasuyama city, Tochigi prefecture, Japan, were examined. We captured the bats under the approval of Governor Tochigi Prefecture. The animals were fixed in 10% formaldehyde solution and dissected under stereoscopic microscope. We identified the brachial plexus and its components through incision of the cervical to thoracic area, and then we dissected toward the distal portion (tip of digits and wing membrane) from the brachial plexus. Furthermore, in order to observe the distribution of these nerves in the wing membrane, Sihler's staining [10] was carried out to dye the nerve fibers a blue-violet color and make the other tissues transparent. The technical terms for the body parts of bats are shown in Fig. 1. The present study was approved by the Committee for Animal Experimentation of the Utsunomiya University.

The brachial plexus was composed of the ventral rami from C5 to T2 (Fig. 2). The components of the radial, the median, and the ulnar nerves, which were distributed to the forelimb and the wing membrane, were restricted to the caudal area of the component of the brachial plexus. The radial nerve was principally formed by C7–T1, the ulnar nerve by C8–T2, and the median nerve by only two, C8 and T1. The ventral rami C5–C7 branched mainly to the shoulder and chest. The axillary nerve to the deltoid muscle was also observed, however the musculocutaneous nerve could not be confirmed under stereoscopic microscope. In the three cases of this study, same composition of brachial plexus and nerve distribution were observed.

The radial nerve ran along the dorsal surface of the humerus, and then to the flexion side near the distal one third of the humerus. This branched into two at the flexion side of the elbow joint (Fig. 3). One branch ran to the dorsal side of the elbow joint cavity and the other ran along the flexion side of the radius-ulna complex and then reached the first finger end along the caudal edge of the propatagium (PRP) (Fig. 4A).

The median nerve ran along the flexion surface of the humerus, and then along the extensor surface of the radius-ulna complex across the ventral side of the elbow joint (Fig. 3). This branched into the five palmar digital nerves at the palmar side of the carpal joint and each branch ran to each finger along the phalanges. It was observed that some thin nerves branched from the median

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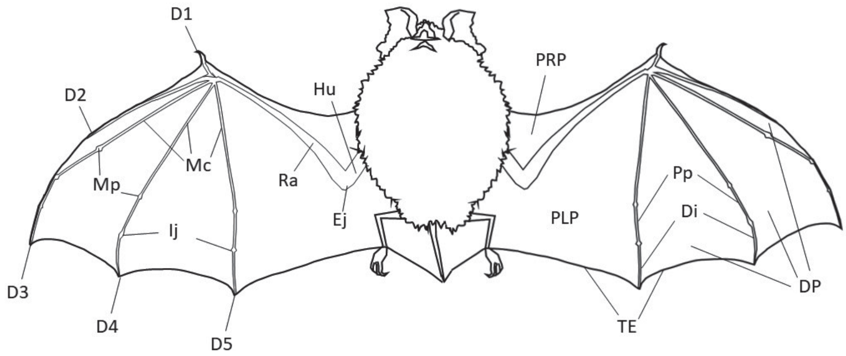

Fig. 1. Technical terms for the body parts of bats. D1~D5, First digit to fifth digit ; Di, distal phalanx ; DP, dactylopatagium; Ej, elbow joint; Hu, humerus; Mc, metacarpal bone; Mp, metacarpophalangeal joint; Pp, proximal phalanx; Ij, interphalangeal joint; PLP, plagiopatagium; PRP, propatagium; Ra, radius-ulna complex; TE, trailing edge.

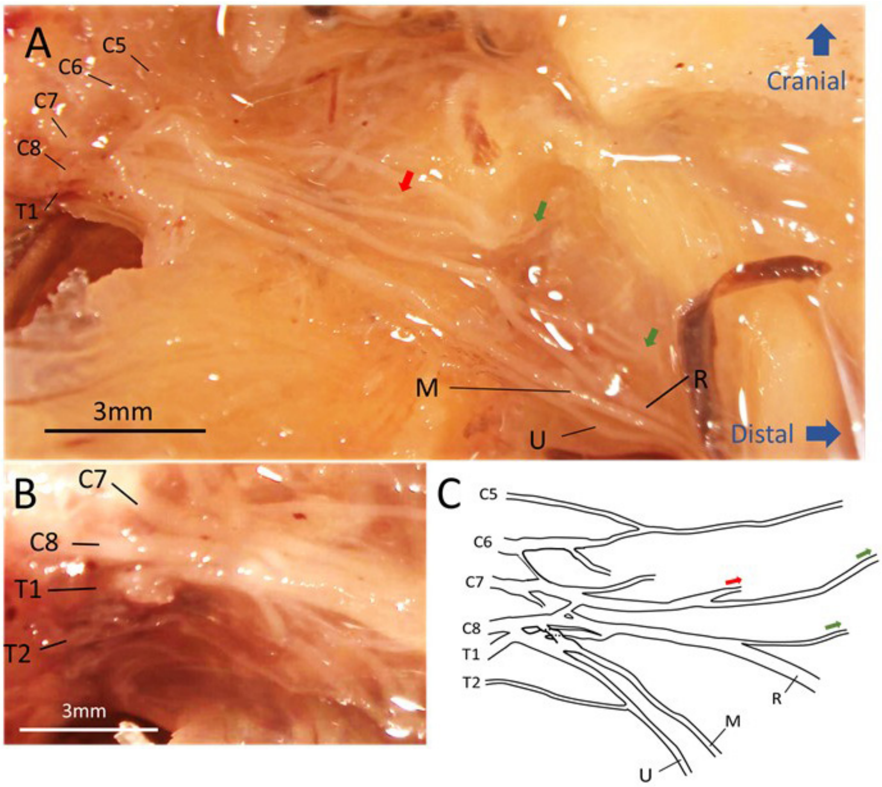

Fig. 2. Composition of the brachial plexus of Japanese little horseshoe bats (A, B) and its schematic diagram (C). Green arrows show the nerve fascicles to the deltoid muscle and red arrows show the nerve fascicles to the serratus anterior muscle. M, median nerve; R, radial nerve; U, ulnar nerve.

nerve, but under the stereoscopic microscope, we could not confirm its innervation area.

Distribution of the branches from the palmar digital nerves in the dactylopatagia (DPs) between the third to fifth fingers was determined (Figs. 4B and 5). All nerves branched out around the metacarpal bone (Mc) and/or the metacarpophalangeal joint (Mp) (Figs. 4B and 5A), although the branching positions varied among specimens. The branches to the DPs terminated near the trailing edge of the wing membrane with some thin branches along the way (Fig. 5B). It was not confirmed under the stereoscopic microscope whether the palmar digital nerve of the second digit distributed branches to the wing membrane.

The ulnar nerve ran along the extensor surface of the humerus and distributed two branches at the elbow joint (Figs. 4C and 6A). One branch continued to the extensor side of the radius-ulna complex, however, it could not be observed beyond a point quarter the length of the radius-ulna complex from its distal end under the stereoscopic microscope. The other branch invaded the plagiopatagium (PLP) at the elbow joint and meandered toward the proximal phalanges of the fifth digit (Figs. 4C and 6B). The

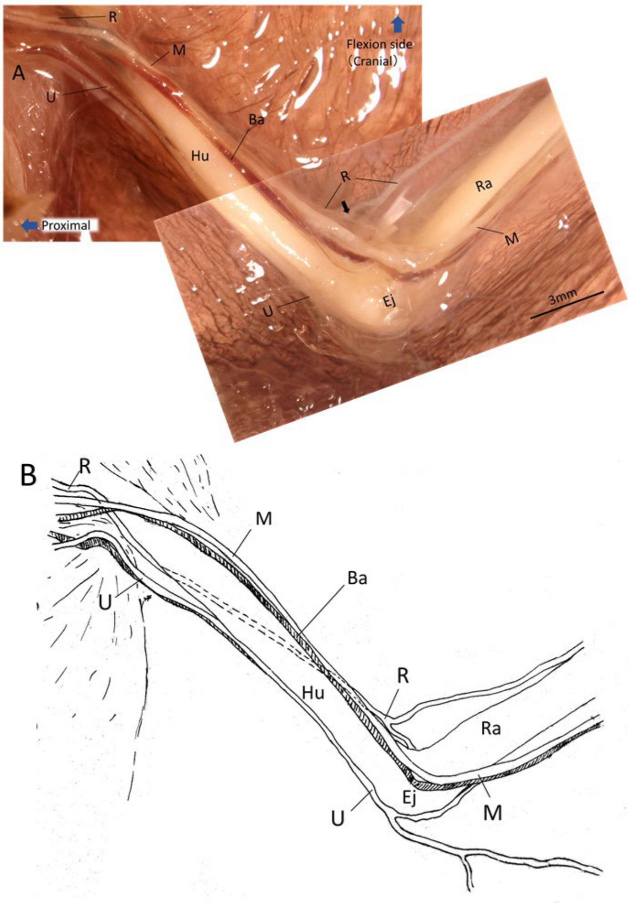

Fig. 3. Ventral view of the left forelimb around the elbow joint in Japanese little horseshoe bats. The nerve running around the elbow joint is shown in the photograph (A) and in the illustration (B). The arrow shows a branch point of the radial nerve. Ba, brachial artery.

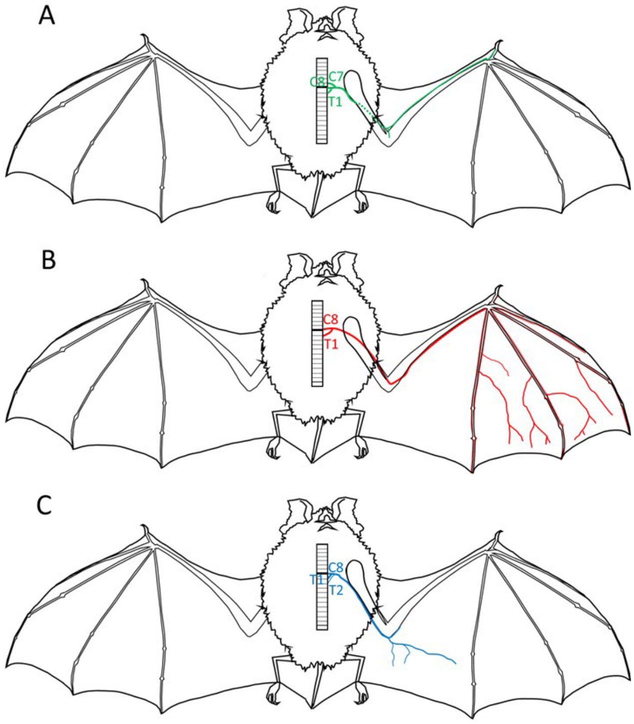

Fig. 4. Distributions and components of the radial (A), median (B), and ulnar nerves (C) in a Japanese little horseshoe bat. This illustration was made based on one specimen.

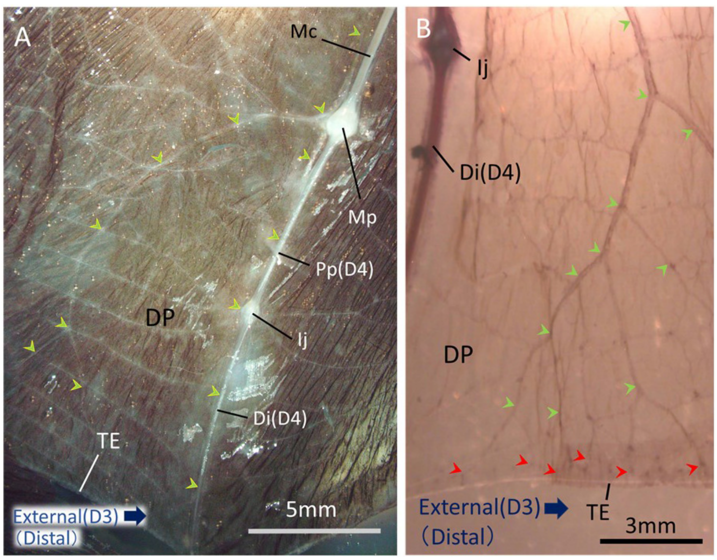

Fig. 5. Nerve running in DPs. The nerve branches in the DP between D4 and D5 is shown in fig. A. The small branches dyed by Sihler's stain in the DP between D3 and D4 is shown in fig. B. Green arrowheads indicate the nerve running and red arrowheads indicate the end of the nerve.

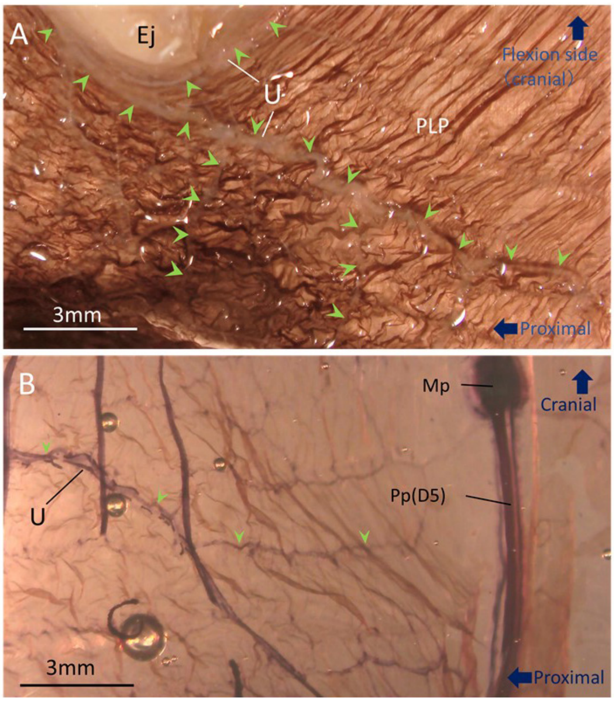

Fig. 6. Ulnar nerve near the elbow joint on left side (A) and its small branches dyed using Sihler's stain in the PLP (B). Green arrowheads indicate the nerve running.

fascicles arising from it diverged repeatedly and ran toward the trailing edge of the PLP.

In this study, we focused on the radial, ulna, and median nerves and their branches that were distributed to the wing membrane. The nerves arising from the first to fifth digit nerves distributed to the DPs (Fig. 4B), indicating that DPs were formed by the extension of finger skin. This theory is consistent with the results of the previous study that the DPs are formed by suppression of apoptosis of the interdigital tissue [13]. The innervation of the ulnar nerve in the PLP (Fig. 4C) also shows that the PLP was formed by extension of the skin from the arm to the fingers rather than that from the thoracolumbar region.

In addition, the innervation area of the ulnar nerve is characteristic of *R. cornutus*. In other mammals, the ulnar nerve usually passes through the forearm, and its branches run to the palmar of the second to fifth metacarpal bones. In *R. cornutus*, however, one of the two branches crossed the stretch side of the elbow joint and only reached the proximal one-fourth of the stretch side of radius-ulna complex. The other one terminated in the PLP and did not reach around the metacarpal bones. On the other hand, the median nerve extended the distribution area to the fifth finger (Fig. 4B). These results indicate that the median nerve replaced the ulnar nerve in the finger areas, and then the main dominant area of the ulnar nerve changed to PLP. In the PLP, there are small muscles that adjust the wing membrane during flight [4]. Therefore, the ulnar nerve may have dominated the sensory receptors and muscles of the skin which are present in PLP.

The components of the nerves forming the brachial plexus have been reported in various animals. For example, the brachial plexus is composed of C6–T1 in horses, *Equus caballus* (Perissodactyla) [6], which through molecular phylogeny are most closely related to bats (Chiroptera) [9], and C7–T2 in dogs, *Canis lupus familiaris* (Carnivora) [3] which can be said to be closely related. Similarly, the brachial plexus is composed of C5–T1 in humans, *Homo sapiens* (Primates) [5], C4–T2 in rats, *Rattus norvegicus* (Rodentia) [1], and C3–T1 in Pacific white-sided dolphins, *Lagenorhynchus obliquidens* (Cetacea) [11]. Thus, the brachial plexus is composed of the ventral rami of the cervical and the thoracic nerves of a total of 4–7 somites. Therefore, the brachial plexus composed of C5–T2 in *R. cornutus* follows the norm. On the other hand, it is notable that the spinal nerves forming the radial, the median, and the ulnar nerves supplying the forelimb and the wing membrane were from a narrow range and placed more caudally than in humans. In humans, it is known that the radial nerve is formed by C5–T1, the median nerve by C5–T1, and the ulnar nerve by C7–T1 [2]. In contrast, in Japanese little horseshoe bats, the radial nerve was mainly formed by C7–T1, the median nerve by C8–T1, and the ulnar nerve by C8–T2. Therefore, it is suggested that more spinal nerves are supplied to the well-developed shoulder and chest muscles for flapping the wings in bats.

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