Axonal mapping of motor and sensory components within the ulnar nerve and its branches

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OBJECTIVE Intrinsic function is indispensable for dexterous hand movements. Distal ulnar nerve defects can result in intrinsic muscle dysfunction and sensory deficits. Although the ulnar nerve’s fascicular anatomy has been extensively studied, quantitative and topographic data on motor axons traveling within this nerve remain elusive.

METHODS The ulnar nerves of 14 heart-beating organ donors were evaluated. The motor branches to the flexor carpi ulnaris (FCU) and flexor digitorum profundus (FDP) muscles and the dorsal branch (DoBUN) as well as 3 segments of the ulnar nerve were harvested in 2-cm increments. Samples were subjected to double immunofluorescence staining using antibodies against choline acetyltransferase and neurofilament.

RESULTS Samples revealed more than 25,000 axons in the ulnar nerve at the forearm level, with a motor axon proportion of only 5%. The superficial and DoBUN showed high axon numbers of more than 21,000 and 9300, respectively. The axonal mapping of more than 1300 motor axons revealed an increasing motor/sensory ratio from the proximal ulnar nerve (1:20) to the deep branch of the ulnar nerve (1:7). The motor branches (FDP and FCU) showed that sensory axons outnumber motor axons by a ratio of 10:1.

CONCLUSIONS Knowledge of the detailed axonal architecture of the motor and sensory components of the human ulnar nerve is of the utmost importance for surgeons considering fascicular grafting or nerve transfer surgery. The low number of efferent axons in motor branches of the ulnar nerve and their distinct topographical distribution along the distal course of the nerve is indispensable information for modern nerve surgery.

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KEYWORDS axon count; flexor carpi ulnaris muscle; flexor digitorum profundus muscle; nerve transfer; deep branch of the ulnar nerve; superficial branch of the ulnar nerve; peripheral nerve; anatomy

NERVE injuries are among the most debilitating neuropathic disorders that can affect otherwise healthy young patients.1,2 Traumatic injury of the ulnar nerve can result in severe impairment of motor function, dysesthesia, and pain in the lesioned hand.3 Surgical intervention is often needed to restore motor and/or sensory hand function in the most acute traumatic injuries. One of the surgical procedures is nerve transfer, whereby branches of uninjured nerves are rewired to injured ulnar nerve branches.4,5 Successful muscle reinnervation is achieved only when the donor nerve axons are correctly wired to the appropriate targets.5,3

The ulnar nerve is responsible for ulnar adduction, finger flexion (digits IV and V), intrinsic hand function as well as sensory innervation of the forearm and ulnar side of the hand. It is divided into superficial and deep branches
at the wrist level, which provide sensory and motor inner-
vation of the hand, respectively. Because of the increasing
feasibility of nerve transfer procedures, various studies
have investigated overall axon counts as well as fascicular
distributions in ulnar nerve branches. \cite{9-11} However, the
distinctive (motor and sensory) axonal composition of these
branches remains scarcely explored, which is attributable
to the lack of specific molecular markers to distinguish
between different neuronal types (i.e., somatic efferent vs
visceral afferent). \cite{12} A recent study has revealed that sensory
components of forearm nerves outnumber motor axons
by a ratio of 9:1. \cite{13} Consequently, distinctive axonal mapping
is missing to provide an accurate blueprint of the axon-
distribution within the ulnar nerve and its branches.

In this study, we performed double immunofluo-
rescence staining of the ulnar nerve and its branches
(deep, superficial, and dorsal branches of the ulnar nerve
[DBUN, SBUN, and DoBUN, respectively]; flexor carpi
unaris [FCU] branch; and flexor digitorum profundus
[FDP] branch). The sensory and motor components of
each nerve branch were quantified. Moreover, the intra-
nuclear distribution of motor fascicles in the distal ulnar
nerve was analyzed.

**Methods**

**Sample Harvesting and Fixation**

Study approval was obtained from the ethics commit-
tee of the Medical University of Vienna. The ulnar nerve
was dissected bilaterally using a standardized approach. \cite{14}
Samples were harvested in 14 heart-beating organ donors
immediately after organ procurement and no more than
12 hours postmortem to prevent enzyme decay (choline
acetyltransferase [ChAT]). First, the bifurcation of the ul-
nar nerve’s main trunk into the superficial (SBUN) and
deep (DBUN) branches was identified in Guyon’s canal.
Afterward, the ulnar nerve was exposed up until the me-
dial epicondyle (Fig. 1) and then followed proximal from
the dorsal branch’s branching point toward the medial epi-
condyle to expose the FCU and FDP motor branches. The
longest branches to the FDP and FCU were harvested for
axon quantification. After harvesting the motor branches,
the SBUN and DBUN were bluntly dissected using optical
magnification until two branches were distinguishable.
After the nerve branches were separated, 2-cm segments
of both the SBUN and DBUN were harvested. Multiple
ulnar nerve specimens were harvested proximal to the bi-
furcation in 2-cm intervals: main trunk distal to proximal
(MT1, MT2, and MT3). The last segment (MT4) was har-
vested 2 cm proximal to the branching point of the dorsal
branch (DoBUN).

The samples were fixed in 4% paraformaldehyde for 24
hours immediately after harvesting, followed by immer-
sion in 0.1 M phosphate-buffered saline (PBS) for the next
24 hours at +4°C. The samples were dehydrated using
increasing sucrose concentrations (10%, 25%, and 40%
in PBS) for 24 hours each and then embedded in Tissue-
Tek O.C.T. Compound (Sakura Finetek Europe B.V.).
The nerve samples were cut into 10-μm-thick sections using a
Cryotome (Leica Biosystems) and mounted on Superfrost
Ultra Plus microscope slides (Thermo Scientific/Menzel).

**Immunofluorescence Labeling**

The harvested samples underwent double immuno-
fluorescence staining using the following neuronal mark-
ers: chicken anti-neurofilament (anti-NF; RRID number
AB_177520, 1:2000), goat anti-ChAT (RRID number
AB_2079751, 1:100). Anti-NF is a pan-neuronal marker,
wheras anti-ChAT visualizes cholinergic axons. \cite{13,15}
Secondary antibodies used in this study were conjugated with
Alexa Fluor 488 or 568 (Thermo Fisher) and used at a
concentration of 1:500.

Before antibody application, the tissue was blocked
with 10% normal rabbit serum for 1 hour. Then, the tis-
ue was incubated with the primary antibodies diluted in
0.1 M PBS containing 0.1% Triton X-100 (PBS-T) for 48
hours at +4°C. Following extensive washing in PBS-T, the
tissue was incubated with the secondary antibodies for 2
hours at room temperature. Finally, the tissue was rinsed
again and mounted using Fluorescence Mounting Medi-
um from Dako (S3023). A more detailed description of
immunolabeling is provided by Gesslbauer et al. \cite{13}

**Confocal and Fluorescence Imaging**

Fluorescence-labeled specimens were analyzed either
with a TissueFAX slide scanner (TissueGnostics) or with
a confocal laser scanning microscope (CLSM; Olympus
FV3000, Olympus Europa SE & Co. KG). A series of
virtual CLSM sections between 1 and 2 μm in thickness
were cut through the structures of interest. Each section
was photodocumented with a 1024 × 1024–pixel reso-
lution, and 3D projections were rendered using ImageJ
software (National Institutes of Health). Double-colored
images were generated using lasers with excitation wave-
lengths of 488 and 568 nm. A semiautomated algorithm
integrated in the software StrataQuest (version 5.1.249)
and TissueQuest (version 4.0.1.0128, TissueGnostics) was
used for axon quantification, as previously described. \cite{13}

**Statistical Analysis**

Normal distribution was tested using the Kolmogorov-
Smirnov test. Descriptive statistics were used for all nerve
samples. Parametric data are presented as absolute and
relative values or as the mean and standard deviation.
Nonparametric values including categorical values are
expressed as the median with interquartile range.

**Results**

**Axonal Components of the Distal Ulnar Nerve Branches**

The main trunk of the ulnar nerve as well as the distal
branches (DBUN, SBUN, and DoBUN) show a polyfas-
cicular architecture (Fig. 1). The ulnar nerve (proximal
to the DoBUN) has an overall axon number of (rounded
mean) 51 (6.4), which are responsible
for motor control of the palmaris brevis muscle. The most

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distal motor branch of the ulnar nerve, the DBUN, has a mixed axonal composition (Fig. 2). Its overall axon number is 8900 (1400), of which 1200 (280) are efferent (Table 1). The quantification indicates that only around 14% of the total neuronal population in the “motor” DBUN actually contribute to motor control of the intrinsic muscles.

Intraneural Fascicular Topography of the DBUN

The motor fascicles of the DBUN and the distal main trunk of the ulnar nerve were identified according to ChAT-positive signals. The overall axon numbers in fascicles containing ChAT-positive signals (motor fascicles of the DBUN) were quantified from distal to proximal along the main trunk of the ulnar nerve up to the DoBUN (Figs. 2 and 3). The overall axon numbers within the motor fascicles are as follows: DBUN 8900 (1400); MT1 10,300 (2500); MT2 11,000 (3500); and MT3 12,200 (3000). The motor axon numbers are as follows: DBUN 1200 (280); MT1 1350 (450); MT2 1350 (420); and MT3 1250 (320). These results indicate constant counts within the motor

![Fig. 1. Fascicular and axonal distribution within the distal ulnar nerve. The main trunk of the ulnar nerve was harvested in segments (MT1–MT4) of 2-cm increments. Cross sections of corresponding ulnar nerve segments represent the distribution of motor (red and yellow axons) and sensory (exclusively red axons) fascicles within the distal ulnar nerve. Illustration © Oskar C. Aszmann, published with permission.](image-url)
fascicles of the ulnar nerve with a slight decrease of the sensory components toward the distal emergence of the DBUN (Fig. 4). Thus, the ratio of motor axons to overall axon number increases from proximal to distal: for MT3, 11% (4.6%); for MT2, 12.5% (4.5%); for MT1, 13.6% (4.2%); and for DBUN, 14.3% (5.3%). The number of motor fascicles varied from distal to proximal: for DBUN, 6.5 (IQR 6–9.5); for MT1, 8.5 (IQR 7.3–10.5); for MT2, 6.5 (IQR 4.8–9.5); and for MT3, 6 (IQR 6–6.8).

Mixed Axonal Composition of the Ulnar Motor Branches

Along with the DBUN, the proximal motor branches (FDP and FCU) of the ulnar nerve demonstrate a mixed axonal composition (Fig. 5). The FDP branch shows an overall axon count of 2100 (370), of which 190 (43) are motor nerve fibers. The FCU branch demonstrated an overall axon count of 1400 (350), of which 140 (61) are motor nerve fibers (Fig. 6). These findings indicate that only a small motor unit number is needed for native motor control of the FCU and FDP muscles.

Discussion

The human hand is the most dexterous biological tool at our disposal. Intrinsic and extrinsic hand functions are governed by the neuronal sources traveling via the ulnar, median, and radial nerves to these muscles. Damage to the ulnar nerve particularly results in severe intrinsic muscle dysfunction and thus has deleterious effects on global hand function. Understanding the distribution and topography of the axonal population within the ulnar nerve is therefore a prerequisite for successful surgical intervention after nerve injury. Here, we reveal the exact distribution of the more than 25,000 axons of the ulnar nerve into its sensory and motor branches at the forearm level. The axonal mapping of more than 1300 motor axons revealed an increasing motor/sensory ratio from the proximal ulnar nerve (1:20) to its deep branch (1:7). The motor branches of the ulnar nerve (FCU, FDP) showed only a share of 10% motor axons, indicating a low motor neuron number responsible for neuromuscular control of the forearm muscles.

Although the ulnar nerve has been intensively investigated because of its clinical importance, previous studies have mainly demonstrated the myelinated axon count or dimensional measurements. A large discrepancy was observed when comparing myelinated axon counts with our total axon counts, highlighting a high number of nonmyelinated fibers in the peripheral nerves. Moreover, myelinated axon counts do not distinguish between motor and sensory axons, thus providing only an approximation of the motor axon population. Distinguishing different axonal types is challenging due to the lack of specific molecular markers for different neuronal types. Recent studies have utilized specific molecular markers to distinguish motor, sympathetic, and afferent axons. This technique allows for the quantitative analysis of motor and sensory axons via the cholinergic metabolism present in all motor fibers.

Modality matched reconstruction is obviously the single most important factor in nerve transfer surgery that influences outcomes by providing a sufficient amount of motor axons reaching their appropriate target muscles while keeping donor site morbidity as low as possible. In 1997, Wang and Zhu described transferring the anterior interosseus nerve (AIN) of the median nerve to the DBUN to restore intrinsic hand function. Schenck et al. analyzed the histomorphometry of the nerves associated with this nerve transfer and revealed that the AIN was significantly smaller in all histomorphometric measurements. Here, the authors showed an overall number of 1800 motor axons in the DBUN, which corresponds to the motor contribution of the ulnar nerve to intrinsic hand function. Knowledge of the exact motor axon number of the DBUN can help surgeons to select the most appropriate donor nerve with the equivalent motor axon number to surgically restore function in low ulnar nerve injuries.

Serial sections of the ulnar nerve in 2-cm increments

### TABLE 1. Absolute axon quantification of motor and sensory components of the ulnar nerve

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall Axon No.</th>
<th>Motor Axon No. (ChAT+)</th>
<th>% Motor Axons</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT4*</td>
<td>26,897 ± 4,966</td>
<td>1,329 ± 296</td>
<td>5.4 ± 1.7</td>
</tr>
<tr>
<td>DBUN</td>
<td>8,910 ± 1,417</td>
<td>1,203 ± 279</td>
<td>14.3 ± 5.3</td>
</tr>
<tr>
<td>SBUN</td>
<td>21,345 ± 3,401</td>
<td>51 ± 6.4</td>
<td>0.24 ± 0.08</td>
</tr>
<tr>
<td>DoBUN</td>
<td>9,306 ± 4,411</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Motor fascicle analysis†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MT1</td>
<td>10,270 ± 2,496</td>
<td>1,352 ± 447</td>
<td>13.6 ± 4.2</td>
</tr>
<tr>
<td>MT2</td>
<td>10,980 ± 3,501</td>
<td>1,346 ± 418</td>
<td>12.5 ± 4.5</td>
</tr>
<tr>
<td>MT3</td>
<td>12,169 ± 3,049</td>
<td>1,245 ± 320</td>
<td>11 ± 4.6</td>
</tr>
<tr>
<td>Motor branches of ulnar nerve</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FDP</td>
<td>2,122 ± 374</td>
<td>191 ± 43</td>
<td>9.1 ± 1.9</td>
</tr>
<tr>
<td>FCU</td>
<td>1,430 ± 351</td>
<td>138 ± 61</td>
<td>9.6 ± 2.8</td>
</tr>
</tbody>
</table>

NA = not applicable.
* The MT4 segment is harvested proximal to the DoBUN.
† Axonal quantification was performed only on the fascicles containing motor axons (ChAT+) within the main trunk of the ulnar nerve.
revealed that the ulnar nerve is arranged into groups of fascicles proximal to its division in Guyon’s canal, which distally become the DBUN and SBUN (Fig. 1). However, this arrangement is not constant over the course of the forearm, as complex rearrangements of fascicles were observed proximal to the ulnar nerve’s division. The finding of a complex fascicular exchange is in accordance with Sunderland’s 1945 report on the topography of the ulnar nerve and has also been described in other nerves as well.\textsuperscript{22,23} In the present study, we outlined the topography of motor axons within the ulnar nerve’s fascicles and showed a varying number of fascicles containing motor axons (6–9) at different levels of the ulnar nerve (Figs. 1 and 4). Furthermore, the share of motor axons in the motor fascicles of the ulnar nerve gradually increased toward the DBUN, which might be explained by interfascicular interchange. This higher motor axon ratio of the motor fascicles in the DBUN compared to the proximal main trunk of the ulnar nerve provides morphological correlates that the most distal coaptation to the DBUN may achieve more precise axonal regrowth toward the target muscles and subsequently a better clinical outcome.

Advances in microsurgery have led to the increasing feasibility of selective nerve transfers, and knowing the anatomy and axon counts of the donor and recipient nerves is essential. Previous reports have demonstrated...
an overall axon count of 832 (218)\textsuperscript{18} or 872 (92)\textsuperscript{24} in the FDP branch and 659 (191),\textsuperscript{18} 372 (35),\textsuperscript{25} or 743 (range 623–900)\textsuperscript{26} in the FCU branch. The higher axon numbers in the present study (2100 for FDP and 1400 for FCU) are attributable the quantification of myelinated as well as nonmyelinated axons. Moreover, our data distinguished for the first time the pure motor axon population (191 in FDP and 138 in FCU) in the motor branches of the ulnar nerve. Knowledge of the precise motor axon numbers in the FDP and FCU branches can facilitate the selection of donor or recipient branches for nerve transfer procedures. Since the motor axons match between donor and recipient nerves is crucial for postoperative outcomes, the data set on the FDP and FCU branches can assist surgeons in the preoperative decision-making on the selection of the most suitable donor/recipient nerve in diverse reconstruction scenarios.\textsuperscript{27–29} While data on the ulnar nerve branches do not provide physicians with a complete spectrum of donor/recipient nerves available for nerve transfers, we aimed to create a comprehensive data set on all peripheral nerve

![share of motor axons in the ulnar nerve](image)

**FIG. 3.** Quantification of the ulnar nerve’s motor and sensory axons. A: Overall axon number of the motor fascicles within the ulnar nerve segments with the corresponding motor axon number. B: Axon quantification of the SBUN, DoBUN, and DBUN. *MT4 corresponds to the ulnar nerve segment proximal to the DoBUN.

![motor axons distribution within the motor fascicles of the ulnar nerve](image)

**FIG. 4.** Motor axons distribution within the motor fascicles of the ulnar nerve. A: The share of motor axons within motor fascicles of the ulnar nerve. B: Number of fascicles in the ulnar nerve containing motor axons. *MT4 corresponds to the ulnar nerve segment proximal to the DoBUN.
branches in the upper extremity. Moreover, these findings showed that only a low number of motor units is needed for natural neuromuscular control of these forearm muscles. This phenomenon grants insight into how much of the neural input should be redirected to a denervated muscle for sufficient functional recovery.

**Limitations**

While immunofluorescence staining of nerve cross sections provided high-quality data that described the afferent or efferent nature of individual axons, curation of these data highly depends on the presence of antigens in the harvested nerves. For this purpose, fresh organ donor nerves were used in this study to achieve reproducible and reliable results. The limited availability of organ donors during the coronavirus disease 2019 pandemic and restricted access were responsible for the small sample sizes; however, standard deviations were generally very small. While staining sequential nerve samples is optimal for axon quantification, its use for studying fascicular arrangements is limited. To overcome this limitation, techniques such as optical tissue clearing should be applied for analysis of the continuous fascicular arrangement.

**FIG. 5.** Axonal topography of the motor branches to the FCU and FDP muscles. The cross sections show a mixed axonal composition of motor (yellow) and sensory (red) axons. Illustration © Oskar C. Aszmann, published with permission.
In the present study, we describe the topographical distribution of 26,900 (5000) total axons in the ulnar nerve at the forearm level and mapped the path of 1300 (300) motor nerve fibers over the course of the forearm until the division into the DBUN and SBUN. A low number of different axons was revealed in the motor branches. Detailed information about the axonal architecture of the human ulnar nerve provides valuable information for surgeons considering fascicular grafting or nerve transfer surgery.

References


**Disclosures**

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

**Author Contributions**

Conception and design: Tereshenko, Maierhofer, Bergmeister. Acquisition of data: Tereshenko, Maierhofer, Hruby, Klepetko, Dotzauer, Laengle, Luft, Festin, Blumer, Bergmeister. Analysis and interpretation of data: Tereshenko, Maierhofer, Dotzauer, Politikou, Laengle, Luft, Festin, Blumer, Bergmeister. Drafting the article: Tereshenko, Maierhofer, Klepetko, Festin, Bergmeister. Critically revising the article: Tereshenko, Maierhofer, Hruby, Politikou, Laengle, Luft, Festin, Blumer, Bergmeister. Reviewed submitted version of manuscript: Aszmann, Tereshenko, Maierhofer, Laengle, Luft, Festin, Blumer, Bergmeister. Approved the final version of the manuscript on behalf of all authors: Aszmann. Statistical analysis: Tereshenko, Maierhofer, Luft. Administrative/technical/material support: Aszmann, Maierhofer, Hruby, Klepetko, Laengle, Bergmeister. Study supervision: Aszmann, Bergmeister.

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