The innervation and organization of motor units in a series-fibered human muscle: the brachioradialis

Zoia C. Lateva, Kevin C. McGill, and M. Elise Johanson

Rehabilitation Research and Development Center, Department of Veterans Affairs Palo Alto Health Care System, Palo Alto, California

Submitted 13 October 2009; accepted in final form 28 March 2010

Lateva ZC, McGill KC, Johanson ME. The innervation and organization of motor units in a series-fibered human muscle: the brachioradialis. J Appl Physiol 108: 1530–1541, 2010. First published April 1, 2010; doi:10.1152/japplphysiol.01163.2009.—We studied the innervation and organization of motor units in the brachioradialis muscle of 25 normal human subjects. We recorded intramuscular EMG signals at points separated by 15 mm along the proximodistal muscle axis during moderate isometric contractions, identified from 27 to 61 (mean 39) individual motor units per subject using EMG decomposition, and estimated the locations of the endplates and distal muscle/tendon junctions from the motor-unit action potential (MUAP) propagation patterns and terminal standing waves. In three subjects all the motor units were innervated in a single endplate zone. In the other 22 subjects, the motor units were innervated in 3–6 (mean 4) distinct endplate zones separated by 15–55 mm along the proximodistal axis. One-third of the motor units had fibers innervated in more than one zone. The more distally innervated motor units had distinct terminal waves indicating tendinous termination, while the more proximal motor units lacked terminal waves, indicating intrafascicular termination. Analysis of blocked MUAP components revealed that 19% of the motor units had at least one doubly innervated fiber, i.e., a fiber innervated in two different endplate zones by two different motoneurons, and thus belonging to two different motor units. These results are consistent with the brachioradialis muscle having a series-fibered architecture consisting of multiple, overlapping bands of muscle fibers in most individuals and a simple parallel-fibered architecture in some individuals.

motor-unit architecture; EMG decomposition; doubly innervated muscle fiber

THE ARCHITECTURAL ARRANGEMENT of the muscle fibers within a muscle and the way in which those fibers are organized into motor units (MUs) are key determinants of the muscle’s biomechanical action, force-generating capacity, and neural control. Although the overall architecture of many human muscles has been studied by dissection and imaging, less is known about the MU architecture of human muscles.

Skeletal muscles can be classified architecturally into pennate muscles, in which the fascicles are short and are oriented oblique to the axis of force generation, and parallel-fibered muscles, in which the fascicles are long and are oriented in parallel to the axis of force generation. In pennate muscles the individual muscle fibers span the length of the fascicle. In parallel-fibered muscles, however, this is not always the case. Many long, straplike animal muscles are made up of serial bands of short fibers that terminate intrafascicularly rather than extending all the way from tendon to tendon (3, 9, 10, 11, 17, 28, 37, 41, 42, 51, 55, 56). One reason for a series-fibered architecture may be to allow a large biomechanical excursion while keeping individual muscle fibers short enough to maintain effective electrical/mechanical coupling.

There is evidence that the human brachioradialis muscle has a series-fibered type of architecture. The brachioradialis has the longest fascicles of any muscle in the human forearm (8, 18, 36). Microdissection studies have shown that it contains non-spanning fibers (7), and staining has shown that it has multiple endplate zones (2) at different proximodistal levels.

Another distinctive characteristic of series-fibered muscles is the presence of a small proportion of muscle fibers with multiple widely separated endplates (3, 19, 56). Unlike the multiple endplates in human facial, tongue, and laryngeal muscle fibers, which are quite close together (typically <150 μm apart) (14, 39, 44, 48), the multiple endplates in series-fibered skeletal muscles can be separated by several millimeters. These fibers are thought to be descended from primary myotubes that extended across multiple innervation zones and received innervation in more than one zone (3).

We previously reported electrophysiological evidence that some muscle fibers in the human brachioradialis muscles have multiple widely separated endplates (25–27). Some MU action potentials (MUAPs) in brachioradialis have a volatile component that fails to appear whenever the MUAP occurs within a few milliseconds after the discharge of another particular MU. The characteristics of the behavior strongly suggest that the volatile component originates from a muscle fiber that is innervated by two different motoneurons, and that the component is blocked by an action-potential collision whenever the two motoneurons attempt to activate the fiber at the same time. According to this hypothesis, the occurrence of blocking should depend on the relative timing of the discharges of the two motoneurons and on the separation between the two endplates. The relationship between endplate location and blocking behavior, however, has not yet been verified.

The way in which muscle fibers are organized into MUs in series-fibered muscles is not fully known. The longitudinal extent of the MU territory is expected to play an important role in the muscle’s biomechanical action (35). Since intrafascicularly terminating muscle fibers transmit much of their force laterally to connective tissue and neighboring muscle fibers, the force ultimately transmitted to the tendon is largely affected by the stiffness or elasticity of the adjacent and in-series fibers (16, 35, 45, 55). One way to achieve effective force transmission would be to arrange a MU’s fibers in a serial fashion so that they act in effect as a single long fiber (52). Series-fibered muscles do receive innervation all along their proximodistal axis to supply the muscle fibers in the different bands (38, 50, 53). However, serial reconstructions have shown that the fibers
of some single MUs in series-fibered animal muscles are confined to a limited proximodistal region (37, 49). This implies that the activation of a single MU may not result in effective force transfer from tendon to tendon and that this can only be achieved as a result of coordinated activation of MUs in different proximodistal bands.

The purpose of this study was to investigate the way in which muscle fibers and MUs are organized in the human brachioradialis. Virtually the only way to study the anatomy of individual MUs in humans is to monitor action-potential propagation using surface or intramuscular electrodes (5, 6, 13, 21, 24, 31, 32, 34, 40, 43, 46). This approach can yield information about the location, extent, and innervation of individual MUs. We used this approach to try to answer the following questions: Does brachioradialis have multiple endplate zones? Are the fibers of individual MUs confined to one proximodistal zone, or are they distributed along the entire length of the muscle? Do some muscle fibers terminate intrafascicularly rather than extending all the way to the tendon? Is the blocking behavior of doubly innervated fibers consistent with the locations of the endplate zones?

METHODS

Subjects. Twenty five subjects (12 women, 13 men, 22–60 yr old, mean 37 yr old) with no history of neuromuscular disorder, diabetes, or orthopedic impairment or surgery of the upper limb participated in this study. The studies were approved by the Stanford University Panel on Medical Human Subjects and conformed to the Declaration of Helsinki. All subjects provided informed consent.

Protocol. Each subject sat comfortably with the arm supported in 15° of elbow flexion (with 0° being full extension) and the forearm in neutral rotation. The brachioradialis muscle was identified by palpation during resisted elbow flexion. The central axis of the muscle was marked from the elbow crease to the palpable distal muscle/tendon junction, and points were marked off along this axis at 15-mm intervals from the elbow crease. (All measurements were given with respect to the elbow crease, with positive indicating distal and negative indicating proximal.) Fine-wire electrode pairs were inserted at 30, 60, and 90 mm. (In two subjects, 10-mm intervals were marked, and fine-wire pairs were inserted at 20, 40, 60, and 80 mm.) These electrodes remained in place throughout the entire experiment to record the same set of MUs during different contractions. The other marked spots along the muscle axis (0, 15, 45 mm, etc.) were sampled one at a time using a monopolar needle electrode. This electrode was inserted at a different spot for each new set of contractions, and the detected MUAPs were matched to the MUs detected by the wire electrodes.

For each location of the needle electrode, the subject performed two 20-s-long isometric contractions by flexing the elbow against resistance provided at the wrist by one of the examiners. These consisted of a “low” isotonic contraction (8 or fewer active MUs per channel, as judged by the signal complexity) and a “moderate” isotonic contraction (8–16 active MUs per channel).

Electrodes. Each fine-wire pair consisted of two 50-μm-diameter stainless steel wires, insulated except for a 1-mm exposed recording surface at the tip (Jari Electrode Supply, Gilroy, CA). The wires were barbed, with one barb 2 mm longer than the other to separate the tips. The needle electrode was a 27-gauge, 37-mm-long needle with 1 mm of exposed recording surface. The electromyographer attempted by feel to position the recording surfaces of the wires and the needle ~5 mm below the fascia covering the muscle, except at the most distal recording sites where the muscle was often thinner than this. The correct positioning of each electrode within brachioradialis was confirmed by testing for the presence of EMG activity during elbow flexion and for its absence during wrist flexion and extension. A reference electrode was placed on the skin surface medial to the 60-mm electrode site. A ground electrode was placed on the back of the hand.

During each contraction, monopolar EMG signals were recorded simultaneously from the needle electrode and each fine-wire electrode with respect to the reference electrode. Note that this resulted in two signals from each fine-wire recording site (one from each wire), which increased the chances of obtaining decomposable signals from those sites. The signals were amplified with filter settings of 5 Hz-5 kHz (Nicolet Viking, Madison, WI), sampled at 10 kHz, and stored on computer. The signals were also high-pass filtered at 1 kHz and displayed in real time to enable the investigators to visualize the signal complexity during the experiment. Audio feedback of the EMG signals was provided to help the subjects maintain steady isotonic contractions.

Signal processing. The EMG signals were decomposed offline into trains of individual MUAPs. This was accomplished by an experienced investigator using the EMGlab computer-aided decomposition program (http://www.emglab.net). The decomposition involved the following steps. First the signal was digitally high-pass filtered at 1 kHz to enhance the MUAP spikes. Then templates were formed for the recurring MUAP spikes, and each discharge in the signal was classified as an occurrence of one or more templates. The program was able to classify many of the discharges automatically, and the rest were classified manually. The investigator checked and edited the results to make sure that the identified firing patterns were smooth and regular and that all the activity in the signal was accounted for.

For each contraction, the MUAP trains detected in the different fine-wire signals were compared, and any time-locked trains (trains whose firing times were offset by a fixed interval from those in another train) were grouped together as belonging to a single MU. Then for each unique MU, MUAP waveforms were averaged from the unfiltered signals from each recording site, using the MU’s identified firing times as triggers. The MUs detected in the fine-wire signals were matched between contractions on the basis of their spike and MUAP waveforms. The final result was a set of MUAP waveforms from each recording site for each MU. Two MUAP waveforms were obtained from each fine-wire site (one from each wire). They typically differed somewhat because of the slightly different locations of the two recording surfaces. The waveform most consistent with the waveforms from the other recording sites was selected for analysis.

Estimation of anatomic parameters. For each MU, the MUAP waveforms from the different recording sites were displayed as a raster plot, as in Fig. 1. The MUAP onset was identified as an abrupt negative- or positive-going departure from the baseline with a characteristic sigmoidal shape. Propagating waves were identified by an increase in the latency of the MUAP spike from one recording site to the next. For each wave, a line was drawn to estimate the propagation of the spike. Many waves appeared in pairs with one wave propagating proximally and the other distally from a common origin. In this case the point at which the lines of propagation intersected was taken as the location of the endplate. Some waves appeared singly, propagating distally from an origin proximal to the most proximal recording site. In this case, the line of propagation was extrapolated proximally, and the point at which it reached a latency of 2 ms after the MUAP onset (the approximate latency at which the peak of the MUAP spike would be expected for a MUAP recorded exactly at the endplate) was taken as the endplate location.

Each MUAP was also manually inspected for the presence of a terminal wave. Terminal waves are small deflections caused by the termination of the action potential at the muscle/tendon junction (24). Terminal waves are positive-going at recording sites between the endplate and the tendon, and negative-going at recording sites beyond the tendon. They are usually seen at more than one recording site at the same latency. If a terminal wave that satisfied these criteria was detected, then the point at which the distal-going line of propagation...
estimated location of the distal termination of the MU. Reached the latency of the peak of the terminal wave was taken as the latency of the terminal wave (open squares). The distal MUAP endplate \((e)\) was estimated from the point at which the distal line of propagation intersected at a point 20 mm distal to the elbow crease, which was taken as the location of the endplates of both MUs. The propagating waves passed out of recording range at 0 mm. The distally going wave of MU 2.1 propagated to 100 mm, where it became a terminal wave at a latency of 17.5 ms. The distally going wave of MU 2.2 propagated to 110 mm, where it became a terminal wave at a latency of 20.4 ms. (Note that the terminal waves were seen as positive-going deflections reaching the latency of the peak of the terminal wave was taken as the estimated location of the distal termination of the MU.

Analysis of doubly innervated fibers. Each signal was checked for MUAP pairs that exhibited interdependent shape irregularity indicative of double innervation (25, 26). The criterion for identifying such pairs was that each MUAP had a volatile component that was blocked or delayed whenever the other MU discharged close before it in time. For each pair, the blocking behavior was characterized by plotting, for each pair of neighboring discharges of the two MUs, the presence or absence of the volatile components as a function of the latency between the discharges (26). The separation between the two endplates was then estimated using the formula \(l = v(w_{1} + w_{2} - 2r)/2\), where \(l\) is the estimated separation (in mm), \(v\) is the propagation velocity (in m/s), \(w_{1}\) and \(w_{2}\) are the widths of the blocking windows (i.e., the maximum latencies for which blocking was observed, in ms), and \(r\) is the refractory period, which was taken to be 3 ms (26).

For those fibers for which it was possible to determine the endplate locations of both parent MUs from the propagation patterns, the endplate separation estimated from the blocking behavior was compared with the value estimated from the propagation patterns.

**RESULTS**

We were able to identify between 27 and 61 (mean 39) distinct MUs in the fine-wire signals per muscle (Table 1). Most of them had detectable spikes at more than one recording site, showing that the electrodes were located along the MU territorial axis. The MUAP spikes recorded by the fine-wire electrodes generally remained stable enough that it was possible to track the same MUs throughout the entire experiment. This made it possible to collect the MUAP waveforms of most of the MUs at the needle as well as the fine-wire recording sites.

**MUAP propagation.** Figure 2 shows the raster plots of two MUAPs from one subject. The onsets of both MUAPs were clearly marked by an abrupt departure from the baseline that was seen simultaneously at several recording sites. The spikes at the different recording sites did not have the same shape and amplitude because the electrodes were located at different relative locations within the MU cross section. Nevertheless, both MUAPs clearly had two propagating waves, one going proximally and one going distally. The propagation velocity of each wave was \(~5\) m/s. For both MUAPs, the lines of propagation intersected at a point 20 mm distal to the elbow crease, which was taken as the location of the endplates of both MUs. The proximally going waves passed out of recording range at 0 mm. The distally going wave of MU 2.1 propagated to 100 mm, where it became a terminal wave at a latency of 17.5 ms. The distally going wave of MU 2.2 propagated to 110 mm, where it became a terminal wave at a latency of 20.4 ms. (Note that the terminal waves were seen as positive-going deflections.

**Table 1. Summary of results**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>MU(s)</th>
<th>Endplate Zones</th>
<th>MU(s) With (\geq 1) Endplate</th>
<th>Doubly Innervated Fibers</th>
<th>Doubly Innervated Branched Fibers</th>
<th>Nonblocked Satellites</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>M</td>
<td>34</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>19</td>
</tr>
<tr>
<td>02</td>
<td>F</td>
<td>36</td>
<td>4</td>
<td>19</td>
<td>11</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>03</td>
<td>F</td>
<td>35</td>
<td>3</td>
<td>14</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>04</td>
<td>F</td>
<td>32</td>
<td>5</td>
<td>14</td>
<td>6</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>05</td>
<td>M</td>
<td>43</td>
<td>5</td>
<td>8</td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>06</td>
<td>M</td>
<td>42</td>
<td>3</td>
<td>23</td>
<td>5</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>07</td>
<td>M</td>
<td>61</td>
<td>4</td>
<td>10</td>
<td>11</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>08</td>
<td>M</td>
<td>39</td>
<td>4</td>
<td>15</td>
<td>6</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>09</td>
<td>F</td>
<td>41</td>
<td>1</td>
<td>0</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>43</td>
<td>4</td>
<td>16</td>
<td>8</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>52</td>
<td>5</td>
<td>29</td>
<td>7</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>32</td>
<td>4</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>32</td>
<td>4</td>
<td>12</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>30</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>28*</td>
<td>6</td>
<td>?</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>46</td>
<td>4</td>
<td>1</td>
<td>12</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>M</td>
<td>46</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>32</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>F</td>
<td>36</td>
<td>3</td>
<td>10</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>F</td>
<td>27</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>F</td>
<td>40</td>
<td>3</td>
<td>9</td>
<td>4</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>22</td>
<td>M</td>
<td>41</td>
<td>4</td>
<td>5</td>
<td>7</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>23</td>
<td>F</td>
<td>37</td>
<td>4</td>
<td>8</td>
<td>7</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>M</td>
<td>38</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>F</td>
<td>32</td>
<td>4</td>
<td>15</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>927</td>
<td>227</td>
<td>119</td>
<td>6</td>
<td>112</td>
<td></td>
</tr>
</tbody>
</table>

*For subject 15, the propagation patterns were too complicated to determine the architecture of the individual motor units (MUs). This subject’s MUs were not included in the MU total. M, men; F, women.
proximal to the point of termination and as a negative-going deflections distal to the point of termination.)

The distal terminations of the two MUs were therefore taken as 100 and 110 mm, respectively. MU 2.2 also had several late components that are described in more detail below. A total of 34 MUs were identified in this muscle. All of them had propagation patterns similar to these; namely, they were all innervated in a single endplate zone and they all extended to the distal end of the muscle.

Figure 3 shows four MUAPs from a different muscle with a more complicated architecture. Each of these MUAPs had a different propagation pattern. MU 3.1 had only a single propagating wave that originated outside the recorded range. Extrapolating the line of propagation to a latency 2 ms after the MUAP onset gave an estimated endplate location of 15 mm.

MUs 3.3 and 3.4 had pairs of propagating waves that originated from endplates at 55 and 80 mm, respectively. MU 3.2 had three propagating waves: a single wave that originated from an endplate at −15 mm, and a pair of waves that originated from an endplate at 40 mm. This MU therefore consisted of two separate groups of fibers. The onset of MUAP 3.2 was 1 ms later at recording sites near the distal endplate than it was at recording sites near the proximal endplate. This reflects the nerve conduction delay between the proximal and distal endplates and corresponds to a nerve conduction velocity of 55 m/s.

Of the four MUAPs in Fig. 3, only MU 3.4 had a distinct terminal wave. It occurred at 12.8 ms, indicating a distal termination at 145 mm. The propagating waves of the other MUs disappeared without detectable terminal waves, suggesting that these MUs terminated intrafascicularly. A total of 32 MUs were identified in this muscle. All the endplates were clustered into four distinct zones centered at 15, 40, 55, and 80 mm. Sixteen MUs had a single endplate at −15 mm, three had endplates at −15 and 40 mm, three had endplates at −15 and 55 mm, six had endplates at 40 and 55 mm, and four had a single endplate at 80 mm. Only the MUs with endplates at 80 mm had detectable terminal waves.

MU organization. The examples shown in Figs. 2 and 3 are illustrative of the MU organization observed in the other muscles studied (Table 1, Fig. 4). In every muscle the endplates were clustered into one or more distinct zones, with the endplates in each zone lying within ±5 mm of one another. The number of endplate zones ranged from one (in 3 muscles) to six (in 1 muscle), with most muscles (18) having three or four. The locations of the endplate zones ranged from −25 mm to 110 mm. The separation between neighboring endplate zones ranged from 15 to 55 mm.

In every muscle with more than one endplate zone, at least one MU had propagating waves originating from more than one endplate zone. Overall, 209 MUs (29%) had waves originating from two endplate zones (such as MU 3.2 in Fig. 3), and 18 (3%) had waves from three endplate zones. In general the onset of the MUAPs recorded near the distal endplate was delayed with respect to the onset of the MUAPs recorded near
the proximal endplate by an amount consistent with a nerve conduction velocity of \( \sim 50 \) m/s.

Not all the MUAPs had detectable terminal waves, and the terminal waves that were detected were not as prominent as in some other muscles (24). Terminal waves were generally detected for MUs in single-endplate-zone muscles (as in Fig. 2), and for the more distally innervated MUs in multiple-endplate-zone muscles (such as MU 3.4 in Fig. 3). The location of the MU termination estimated from the latency of the terminal waves corresponded roughly to the palpated distal end of the muscle. For the more proximally innervated MUs in multiple-endplate-zone muscles the propagating waves usually disappeared before reaching the distal end of the muscle without a detectable terminal wave, as for MUs 3.1–3.3 in Fig. 3.

**Depth.** Even though the latencies of most of the detected terminal waves were consistent with MU terminations at the palpable distal end of the muscle, a number of MUs had terminal waves with latencies that indicated terminations up to 50 mm more proximal than the distal end of the muscle. One possible explanation is that these were deeper MUs that terminated onto the aponeurosis of the distal tendon at sites more proximal than the distal end of the muscle. To explore this idea, in two muscles we made recordings at two different depths below the skin surface. Specifically, for each needle insertion we recorded signals at two different depths, one more superficial and one \( \sim 5 \) mm deeper. Figure 5 shows the propagation patterns of two MUs from one of these muscles. The upper sets of traces show the MUAPs that were recorded more superficially, and the lower sets show the MUAPs that were recorded...
components were detected at the 0-, 15-, and 105-mm recording sites (Fig. 6B). The blocking behavior is shown in Fig. 6C. The widths of the combined blocking windows (w1 + w2) were 22 ms for each fiber, giving an estimated separation between endplates of 27 mm, which agrees well with the distance estimated from the propagation patterns.

The blocking behavior at each recording site was also consistent with the location of the recording site with respect to the endplates. The action potential from one endplate of a doubly innervated fiber will be blocked if it collides with an action potential from the other endplate before reaching the electrode or if it fails to be initiated because the endplate is refractory after the passage of an action potential from the other endplate. The electrode at 0 mm was approximately midway between the two endplates, and so it saw the volatile components of both MUs block approximately equally. The electrode at 15 mm was also between the endplates, but very close to the endplate of MU 6.2, and so it saw the volatile component of MU 6.2 blocked only for intervals equal to and slightly longer than the refractory period. The electrode at 105 mm was distal to both endplates, and so it saw the volatile component from MU 6.2 blocked only during the refractory period. The volatile component from MU 6.2 was also delayed by up to 5 ms at the 105-mm recording site during the relative refractory period following an action potential from MU 6.1. This is consistent with a decreased propagation velocity and a long conduction path between the endplate and the recording site.

For some of the MUAP pairs it was not possible to estimate the location of both endplates from the propagation patterns. An example is shown in Fig. 7. These two MUs shared a doubly innervated fiber that was seen at the 75-mm recording site. In this case, the propagation pattern of MU 7.1 indicated an endplate location at 65 mm. However, MU 7.2 was only detected at two sites, and thus it was not possible to determine the location of the endplate of MU 7.2 reliably from its propagation pattern. The blocking behavior (Fig. 7B) predicted an endplate separation of 80 mm. This would place the endplate of MU 7.2 at about −15 mm, which corresponded to an endplate zone at which several other MUs in this muscle were innervated.

In general, there was good agreement between the blocking windows and the estimated endplate zone locations for all the identified doubly innervated fibers, as in these two examples. Thirty-two of the doubly innervated fibers were innervated at neighboring endplate zones, and the other 87 were innervated two or three endplate zones apart. The largest observed separation between the endplates of a doubly innervated fiber was 80 mm.

Doubly innervated branched fibers. An additional six MUAP pairs exhibited volatile behavior that was not consistent with a single doubly innervated fiber. These MUAPs had volatile satellite potentials whose blocking behavior was consistent with a doubly innervated branched fiber. Branched fibers have two separate branches that are in electrical continuity at some distant point. An action potential that passes the electrode on one branch propagates to the branching point and then back along the other branch, repassing the electrode as a satellite potential. In these six MUAP pairs the satellite potentials sometimes failed to appear, indicating double innervation. These pairs have been described in detail elsewhere (25).
Two of the volatile satellite potentials can be seen in MUAP 2.2 of Fig. 2 as the potentials marked a. The behavior of both potentials was affected by the proximity of the nearest discharge of another particular MU. The latencies of both potentials were consistent with distal propagation from the endplate of MU 2.2 to a branching point located at ~70 mm and then proximal propagation back to the electrode (dashed gray line). Since these two potentials were recorded at two different sites, we cannot be sure whether they came from the same branched fiber, or from two different branched fibers with a similar branching point. Three of the doubly innervated branched fibers were found in single-endplate-zone muscles and the other three were found in multiple-endplate-zone muscles.

Satellite potentials. MU 2.2 in Fig. 2 had two additional satellite potentials (marked b) that were never blocked. These potentials could have come from branched fibers that were not doubly innervated (23) or from doubly innervated branched fibers whose other coinnervating motoneurons were not recruited during the recorded contractions. The latencies of the potentials indicate a conduction distance of ~80 mm, which would imply a branching point at either 60 or ~20 mm. A total of 112 nonblocked satellite potentials were found across all the muscles (Table 1).

**DISCUSSION**

This study investigated the anatomic organization of MUs in the human brachioradialis muscle by analyzing MUAP waveforms recorded intramuscularly at different sites along the muscle axis. From the MUAP propagation patterns we were able to determine the location of the motor endplates and the distal muscle/tendon junctions.

Propagation patterns. For each MU we compiled a raster plot of the MUAP waveform at evenly spaced sites along the muscle axis. If the MU’s endplate was located within the span of the electrodes, the raster plot showed two waves, one propagating toward the distal end of the muscle and one toward the proximal end. In this case the location of the endplate was estimated by finding the common origin of the two waves. If the endplate was more proximal than the most proximal electrode, the raster plot showed only a single wave. In this case the endplate location was estimated by extrapolating the propagation back to the time at which the peak of the MUAP spike would have occurred at the endplate, namely, ~2 ms after the MUAP onset. The MUAP onset was clearly marked for most MUs by an abrupt negative-going departure from the baseline (at sites very near the endplate), or by an abrupt positive-going departure with a characteristic sigmoidal shape (at sites farther away).

The point of termination of the propagating waves was not always easy to determine. For some MUs the termination was marked by a distinct terminal wave—a standing wave produced by a change in the dipole moment of the transmembrane currents when the action potential terminates at one end of the muscle fibers. Terminal waves were seen as small deflections that occurred at the same latency at more than one recording site. The location of the termination was estimated by extrapolating the propagating wave to the latency of the terminal wave (e.g., MU 3.4 in Fig. 3). In some cases the raster plots showed propagation up to the point of termination but not beyond (e.g., MU 2.1 in Fig. 2), which provided an additional confirmation of its location.

For many MUs, however, the propagating waves disappeared before reaching the distal end of the muscle without leaving a detectable terminal wave (e.g., MUs 3.1–3.3 in Fig. 3). One possible explanation is that the distal path of these MUs fell outside the pick-up range of the electrodes. This is unlikely, however, since other MUs in the same muscles were well seen by all electrodes. More likely, these MUs terminated intrafascicularly before reaching the distal end of the muscle. In this case the terminal waves were attenuated because the ends of the individual fibers were spread out over a relatively long distance or because of fiber tapering (20). However, since the amplitude of the intramuscularly recorded MUAP is not a reliable gauge of the total number of fibers that reach a
particular proximodistal level, the precise characteristics of the intrafascicular terminations could not be estimated from the propagation patterns.

The overall picture presented by the propagation patterns is that the MUs innervated at more distal endplates (including the MUs in single-endplate-zone muscles) extended to the distal tendon, whereas the MUs innervated more proximally terminated intrafascicularly before reaching the distal tendon. The same was probably also true at the proximal end of the muscle: the MUs innervated close to the proximal end originated from
the tendon, while those innervated more distally originated intrafascicularly.

It should be mentioned that the MUAP onsets and terminal waves were not as well seen in brachioradialis, especially in the muscles with multiple endplate zones, as they are in some other muscles such as biceps brachii and tibialis anterior (24). Since the amplitude of the onset and terminal wave depend on the innervation ratio (i.e., the total number of muscle fibers in the MU), the smaller amplitudes may reflect lower innervation ratios in brachioradialis compared with the other muscles.

Multiple endplate zones. This study confirmed that most human brachioradialis muscles have multiple endplate zones along their proximodistal axis. In each muscle the endplates were clustered into distinct zones no more than \( \leq 10 \) mm in longitudinal extent. A majority (22 of 25) of the muscles had more than one endplate zone. Most had three or four endplate zones, with the most proximal zone being near the elbow crease and the most distal ranging from 30 to 110 mm distal to the crease. Even though we did not place electrodes proximal to the crease, it was possible to estimate the locations of some proximal endplates by extrapolation (e.g., MU 3.1 in Fig. 3). These results are in general agreement with the cholinesterase staining study of Christensen (2), who found the endplates in the brachioradialis muscles of stillborn infants to be distributed in two zones, one in the middle of the muscle and one more distal.

Our results also indicate, however, that the existence of multiple endplate zones is not a universal feature of the human brachioradialis. In three muscles (1 from a female subject and 2 from male subjects) we were only able to detect a single endplate zone. To check whether this could have been due to inadequate sampling, we analyzed signals from several additional cross-sectional locations in one of the muscles but failed to detect any other endplate zones. We are therefore fairly confident that this one muscle, and probably the other two as well, had only a single endplate zone.

Anatomic studies of human cadavers have shown that the brachioradialis is innervated by from 1 to 4 (mean 2.7) extramuscular branches of the radial nerve at entry points separated by as much as 50 mm (1, 22, 47). It is not unreasonable to think that these distinct branches innervate the distinct endplate zones. The long neck muscles of the cat similarly receive innervation by several distinct nerve bundles that enter the muscle at different rostrocaudal levels (42). The most distal
endplate zones we observed were considerably more distal than the most distal entry points reported by Latev and Dalley (22). Thus the more distal nerve branches may travel some distance after entering the muscle before reaching their endplate zones. It should also be noted that 4 of the 43 muscles reported by Latev and Dalley were innervated by only one nerve branch, which is about the same percentages as the number of muscles we found to have only one endplate zone.

Longitudinal distribution of the fibers of individual MUs. Our results clearly show that some brachioradialis MUs have muscle fibers in different proximodistal bands. The MUAPs of these MUs showed propagating waves originating from two and, in a few cases, three different endplate zones (e.g., MU 3.2 of Fig. 3). The largest separation between endplate zones innervated by the same motoneuron that we observed was 80 mm, which is comparable to findings in the human sartorius muscle (15).

We were able to estimate the conduction velocity of the axonal branch to the distal endplate from the difference in latency of the MUAP onset at more proximal and more distal sites. This difference gave an estimated velocity of ~50 m/s, which implies that the branch was myelinated. If the different endplate zones are indeed supplied by separate external nerve branches, then the axonal branching presumably took place in the main nerve trunk (4, 54) rather than within the muscle.

For the majority (68%) of MUs, however, the propagation patterns only showed signs of a single endplate zone. This is consistent with serial reconstructions in series-fibered animal muscles, which have shown that the fibers of some single MUs are confined to a limited proximodistal region (37, 49). Some of the MUs in our study could have had additional groups of fibers in another part of the muscle cross section that escaped detection. Nevertheless, it seems clear that the MUs in brachioradialis are not universally arranged into serially linked bands as in the single-long-fiber hypothesis (52). This implies that the nervous system must recruit MUs in each fiber band in a coordinated way to ensure effective force transmission.

Doubly innervated muscle fibers. An interesting feature of series-fibered muscles is that some of their muscle fibers have more than one endplate and receive innervation from more than one motoneuron. We found two different types of doubly innervated fibers in brachioradialis. The most common type were single fibers innervated by two different motoneurons in two different endplate zones. We found such fibers in every muscle that had multiple endplate zones. These fibers were recognized when two MUAPs in the same signal had identically shaped components that were delayed or blocked whenever the two MUs discharged close together in time. Blocking occurred whenever the action potentials from the two endplates collided and one of them failed to reach the electrode. Delays occurred whenever an action potential was initiated at one endplate during the relative refractory period after the passage of an action potential from the other endplate. We previously showed that the window of separation between the discharges of the two MUs over which blocking occurs should be related to the distance between the two endplates (26). The results of the present study confirm that this is indeed the case and that the blocking behavior is consistent with the locations of the endplate zones within the muscle.

The fact that some doubly innervated fibers crossed three or more endplate zones suggests that they are longer than the intrafascicularly terminating fibers. For some MUs the only component we were able to detect was a doubly innervated fiber, implying that this fiber extended far beyond the main MU territory. It is likely that some of the stable MUAP components we recorded came from doubly innervated fibers whose other coinnervating motoneuron was not recruited during the recorded contractions. This may have led to overestimation of the main territorial extent of these MUs.

The second type of doubly innervated fibers we observed were branched fibers. These were fibers in which an action potential propagated in one direction to a branching point and then back in the opposite direction along the other branch. The potential passed the electrode twice, first as part of the main MUAP spike and then as a satellite potential (25). The satellite potentials were sometimes blocked because of collisions with action potentials from the other endplate. Compared with the doubly innervated single fibers, these fibers were relatively rare. We found only six doubly innervated branched fibers in all. Unlike the doubly innervated single fibers, three of these were found in muscles that had only one endplate zone. In these cases, both branches were innervated by different motoneurons in the same endplate zone.

Series-fibered architecture. This study confirms that the human brachioradialis has the characteristics of a series-fibered muscle. It has multiple endplate zones, MUs that terminate before reaching the distal end of the muscle, and doubly innervated muscle fibers. The endplate zones are distributed unevenly along the length of the muscle, unlike the case in series-fibered muscles of the mouse, guinea pig, and rabbit (38). The bands of fibers innervated in each endplate zone overlap considerably, extending beyond the neighboring innervation point. There is significant variability between subjects both in the number and location of the endplate zones (Table 1). Similar complexity and variability of MU architecture has also been reported for the human and macaque gracilis muscle (38).

It is worthwhile to consider how the results of this study relate to the overall three-dimensional architecture of the muscle. The dissection study of Fridén et al. (8) found fascicle lengths in brachioradialis to vary from ~180 mm superficially to ~100 mm inferiorly. Our recordings were made in the superficial fascicles in a volume ~10 mm wide and 5–10 mm deep along the midline of the muscle. Since we sampled 10 or more separate sites per subject, we are confident that the results provide an accurate description of the innervation and motor-unit organization in this cross section. We recorded from deeper fascicles in two subjects. Those recordings showed that the deeper MUs terminate much more proximally on the distal aponeurosis than do the more superficial ones, consistent with Fridén et al. The deeper recordings further suggest that the deeper fascicles may contain only a single band of fibers even when the more superficial fascicles are series-fibered. Although we did not sample extensively in the medial-lateral direction, we would expect the motor-unit organization to be similar: more apt to be series-fibered in longer superficial fascicles and more apt to be tendon-to-tendon in the shorter, deeper ones.

A series-fibered architecture allows a muscle to have a large excursion while being made up of relatively short fibers (28, 38). It has been suggested that if muscle fibers are too long, then the relatively slowly propagating MUAP may not reach
the distant sarcomeres quickly enough to ensure a synchronous, mechanically efficient activation of the entire fiber (16, 28, 56). According to this argument, muscle fibers are most efficient if they are short enough so that the MUAP propagation time from endplate to tendon is a small fraction of the twitch contraction time. However, it is not clear that the intrafascicularly terminating fibers in the human brachioradialis achieve this goal. For human muscles, twitch contraction times are generally in the range of 63–75 ms (12, 29, 30). Although most of the MUAPs in our study had propagation times of <25 ms, some had propagation times as long as 36 ms (e.g., MU 6.1 in Fig. 6). This is half the expected twitch contraction time and would seem to violate the short-fiber argument. It is also not clear why a series-fibered architecture might be necessary for some individuals but not for others. These points deserve further study.

ACKNOWLEDGMENTS
We thank Wendy Murray and Vincent R. Hentz for helpful discussions.

REFERENCES


