Form and classification of motor endings in mammalian muscle spindles

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(Communicated by J. Z. Young, F.R.S. – Received 2 January 1985)

[Plates 1 and 2]

The presynaptic features of 234 motor endings supplied to cat hindlimb muscle spindles have been studied in teased, silver preparations, and the postsynaptic features of a further 27 endings have been studied in serial, 1 µm thick, transverse sections. In the presynaptic study motor endings received by the three types of intrafusal muscle fibre were compared with the endings supplied to spindles by the various functional categories of motor axon. Three forms of motor ending were found that had significantly different presynaptic features. These forms correspond closely to those previously identified in the literature as p1 (β), p2 (dynamic γ) and trail (static γ). The results of the postsynaptic study showed that the degree of indentation of the intrafusal muscle fibres by motor axon terminals increases with greater distance from the primary ending, irrespective of muscle-fibre type. We conclude that the postsynaptic form of intrafusal motor endings is determined by distance from primary ending and muscle-fibre type. It is not determined by type of motor axon, and cannot be correlated with presynaptic form so as to produce a unified classification of intrafusal motor endings.

Introduction

The classification of mammalian intrafusal motor endings into two types of plate (p1, p2) and trail endings, described in detail by Barker et al. (1970), was based on an analysis of the presynaptic features of the endings as seen in teased, silver preparations of cat muscle spindles. It was shown that the three categories could further be distinguished by their different rates of degeneration after nerve section. The same types of motor ending were recognized in silver-stained spindles of other mammals, namely, rat (Gladden 1969), rabbit (Barker & Stacey 1970; Barker et al. 1972), and man (Kennedy 1970; Swash & Fox 1972). At the time this work was carried out mammalian spindles were generally thought to be composed of two types of muscle fibre (nuclear-bag and nuclear-chain fibres), and discussion centred on how these two types were innervated by the functional categories of dynamic and static γ axons (Matthews 1972).

Since that time a number of important advances have occurred that make it necessary to re-examine the p1, p2, trail classification. It is now accepted that there are three kinds of mammalian intrafusal muscle fibre (bag1, bag2, and chain fibres),
196 R. W. Banks, D. Barker and M. J. Stacey

and that dynamic actions are carried out by the bag_1 fibre (the dynamic bag fibre) and static actions by the bag_2 (static bag fibre) and chain fibres. The manner in which β and γ motor axons are distributed to these fibre types in cat spindles is now generally agreed (see reviews by Boyd 1981; Barker & Banks 1985). Chain fibres are now referred to as ‘long’, ‘intermediate’, or ‘typical’ in terms of their length relative to the capsule (Barker et al. 1976a; Kucera 1980a, 1982a). These subtypes are of functional significance since in cat spindles there is a selective innervation of long and most intermediate fibres by static β axons (Harker et al. 1977; Jami et al. 1979) and of typical chains by static γ axons (Barker et al. 1976b).

Finally, detailed information has become available about the sensory innervation of cat spindles (Banks et al. 1982) that can be used in identifying intrafusal muscle-fibre types in teased, silver preparations and so assist in the analysis of their motor innervation.

In re-examining the presynaptic features of cat intrafusal motor innervation our approach was to study the motor endings received by the various types of intrafusal muscle fibre, and to compare these with the endings supplied to spindles by the various functional categories of motor axon. We found three forms of motor ending that had significantly different presynaptic features. These forms correspond closely to those described in the p_1, p_2, trail classification.

When the postsynaptic form of cat intrafusal motor endings is considered, the question arises: is it mainly determined by the type of motor axon that supplies the endings, or by the type of muscle fibre that receives it? Barker’s group initially held the former view (Barker et al. 1970), but later work convinced them that muscle-fibre type and distance of the ending from the equator were the determining factors (Barker et al. 1976a; Barker et al. 1978). Recent studies by Banks (1981, 1983) and Kucera (1980a, b, 1981, 1982a, b) have provided evidence in support of this. Meanwhile ultrastructural studies by Arbuthnott et al. (1982) have led them to propose a new classification of cat intrafusal motor endings in which all are regarded as plates. Five types of plate are recognized mainly by the degree and manner of the indentation of their axon terminals into the muscle-fibre surface. Type of plate is seen as being determined either by type of muscle fibre (for example, as in the case of ‘m_b plates’ supplied to bag_1 fibres by dynamic β and dynamic γ axons), or by type of motor axon (for example, as in the case of ‘m_d plates’ supplied to long chain fibres by static β axons).

In this paper we examine both presynaptic and postsynaptic features of cat intrafusal motor endings, and discuss whether it is possible to correlate these so as to produce a unified classification. Preliminary accounts of some of the results have been published (Banks 1983; Barker & Stacey 1984; Banks et al. 1985).

**Material and Methods**

*Silver-stained spindles*

Presynaptic features of the motor endings received by the various types of intrafusal muscle fibre were studied in spindles teased from the peroneus brevis and tenuissimus muscles of normal cats. For the study of endings belonging to the different functional types of motor axon we were able to use muscles from previous
work in which chronic degeneration experiments had been carried out to isolate single surviving static γ axons (Barker et al. 1973) or β axons (Barker et al. 1980). We also used peroneal muscles from which the β innervation had been eliminated by degeneration for 54 h after nerve section. Some of these muscles had been prepared by Barker et al. (1970), others were similarly prepared for the present study. The silver technique used was either that described by Barker & Ip (1963), or the modification of this reported by Barker et al. (1984). Full details regarding the material used are given in table 1.

**Table 1. Silver-stained material studied**

<table>
<thead>
<tr>
<th>source</th>
<th>number of muscles</th>
<th>number of spindles</th>
<th>number of motor endings</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal muscles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>peroneus brevis</td>
<td>4</td>
<td>11</td>
<td>76</td>
</tr>
<tr>
<td>tenuissimus</td>
<td>4</td>
<td>5</td>
<td>32</td>
</tr>
<tr>
<td>differentially degenerated muscles:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>surviving spindle motor axons:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>static γ tenuissimus</td>
<td>5</td>
<td>15</td>
<td>76</td>
</tr>
<tr>
<td>tenuissimus</td>
<td>3</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>superficial lumbrical</td>
<td>2</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>abductor digiti</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>quinti medius</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>γ peroneus brevis</td>
<td>3</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>peroneus longus</td>
<td>2</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>peroneus tertius</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong>totals</strong></td>
<td><strong>26</strong></td>
<td><strong>64</strong></td>
<td><strong>234</strong></td>
</tr>
</tbody>
</table>

For the purposes of this study we have regarded a motor ending as a discrete group of axon terminals supplied by one or more preterminal axons to the polar region of a single intrafusal muscle fibre. We have interpreted 'discrete' to mean that the terminals are supplied to part of the muscle-fibre surface within which there is no portion free of terminals for more than a length of 10 μm. This definition allows for an ending to be supplied by more than one parent axon, but this rarely occurred in our sampling.

In silver-stained material it is not possible to decide precisely where the transition from preterminal axon to axon terminal occurs. Hence as part of the attempt to quantify motor-ending form we measured the total length of unmyelinated axon present in each ending, and designated this measurement ‘PTL’ (that is, preterminal plus axon terminal length). The measurement was made from the origin of the preterminal axon at the heminode of the supplying axon, to the end of each axon terminal. In those instances where the preterminal axon branched to provide a further ending, the origin of its preterminal axon was regarded as the branch point from which the further ending was supplied (see figure 1c). Measurements of PTL were made with a map-measuring wheel on tracings prepared by using a Nikon Optiphot with a × 100 objective coupled to a television projection system. Since the tracings formed a projection plan of the ending the transverse component of the PTL was reduced. This would have been
Figure 1. (a)–(c) Tracings of silver-stained preparations of motor endings from cat peroneus brevis spindles to illustrate examples of measuring ending length \((L)\), the ending being regarded as including one or more preterminal axons and their axon terminals. In each tracing the ending is shown black, and the myelinated axon that supplies it is unshaded. The endings were located on a bag_1 fibre \((a)\), a chain fibre \((b)\), and two chain fibres \((c)\). The disposition of the endings in \((c)\) is unusual. \((d)\) Diagram to illustrate the terminal profile presented by a motor axon terminal (hollow outline) embedded in the surface of an intrafusal muscle fibre (stippled) as seen in a 1 \(\mu\)m thick transverse section of a cat spindle stained with toluidine blue. Arrowed lines indicate measurements made of the maximum width \((x)\) and depth \((z)\) of the terminal and its indentation \((y)\) into the muscle-fibre surface.

relatively more important in richly branched endings such as the most complex of those supplied to the bag_1 fibre. Consequently the observed difference between these and less complex endings will have been artificially reduced.

We also measured the length \((L)\) of each ending parallel to the longitudinal axis of the muscle fibre (figure 1a–c); since \(L\) included preterminal axon it would usually have exceeded the length of the actual neuromuscular junction. Other data recorded for each ending were number of axon terminals, and location with respect to the spindle regions A, B, and C defined by Barker et al. (1976a). Finally, the internodal diameter of the parent axon was measured (excluding myelin), and the number of times it branched within the spindle was counted.

The three types of intrafusal muscle fibre, and the subtypes of chain fibre, were identified on the basis of their length and diameter, and details of their sensory innervation. When sensory endings were absent owing to their degeneration together with the \(\beta\) innervation after nerve section, bag-fibre types were distinguished mainly by the scarcity (bag_1) or abundance (bag_2) of elastic fibres associated with them (Gladden 1976).

Serially sectioned spindles

Serial, 1 \(\mu\)m thick, transverse sections of 27 tenuissimus intrafusal motor endings containing 442 axon terminals were examined to study the relationships made between their terminals and the underlying muscle-fibre surface. The endings
formed part of the sample studied by Banks (1981) and comprised all those supplied to the proximal poles of spindles 6 and 9 in that study, as well as those supplied to the distal pole of spindle 12. Enlarged photographs (×1800) of 3189 terminal profiles were used to measure, to the nearest 0.5 mm, the maximum width (x) and depth (z) of each axon terminal and its indentation (y) into the muscle-fibre surface (see figure 1d). Sampling of several endings for electron microscopy confirmed that individual terminals were readily identifiable in the light-

intrafusal motor endings in normal muscle

on bag₁ fibres

on long and intermediate chain fibres

on typical chain fibres

on bag₂ fibres

endings of intrafusal motor axons in experimental muscle

endings of Y axons on bag₁ fibres

endings of β axons on bag₁ fibres

endings of static Y axons on bag₂ and typical chain fibres

Figure 2. A sample of cat intrafusal motor endings traced from silver preparations teased from normal and experimental muscles (mainly tenuissimus and peroneus brevis). In each tracing the ending (that is, the preterminal axon and axon terminals) is shown black, and the myelinated axon that supplied it is unshaded.
microscopical preparations (plate 2). Thin Schwann-cell processes covering the terminals could not be separately resolved in the photographs, but their thickness is considerably less than the error inherent in each measurement.

Three ratios were obtained from the data, namely, eccentricity \( (E = x/z) \), indentation \( (I = y/x) \), and superficiality \( (S = y/z) \). The ratios varied widely within and between individual terminals of each ending, but standard errors of the mean values for complete endings were small due to the large amount of data (see figure 7). The number and length of terminals in each ending were also recorded as well as the number of nuclei in its sole plate. Finally, the location of a motor ending on a given muscle fibre with respect to the primary ending was recorded as the distance between the midpoint of the ending's neuromuscular junction and the midpoint of the primary terminals supplied to the fibre.

Most of the data from both the silver and serial-section analyses were clearly not normally distributed. We therefore used a non-parametric method of comparison, namely, Wilcoxon's rank sum test for a two-sample comparison (Bailey 1981).

\[
\begin{align*}
\text{length/\mu m} & \quad 40 \quad 80 \quad 120 \quad 160 \quad 200 \quad 240 \\
\text{number of endings} & \quad 2 \quad 4 \quad 6 \quad 8 \quad 10 \quad 12 \\
\text{preterminal plus axon terminal length/\mu m} & \quad 80 \quad 160 \quad 240 \quad 320 \quad 400 \quad 480
\end{align*}
\]

**Figure 3.** Histograms of \( L \) and PTL measurements of cat intrafusal motor endings. These show that the endings supplied to bag_1 fibres in normal spindles (a) consist of different populations belonging to \( \gamma \) (b) and \( \beta \) (c) axons; and that the \( \beta \) endings are very similar to those supplied to long and intermediate chain fibres (d). The endings measured in (a) and (d) belonged to spindles in normal peroneus brevis and tenuissimus muscles; those measured in (b) were supplied to bag_1 fibres by \( \gamma \) axons that remained after the \( \beta \) endings had degenerated in peroneal muscles following nerve section; and those measured in (c) belonged to \( \gamma \) axons that had survived in the chronic degeneration experiments of Barker et al. (1980) on tenuissimus, superficial lumbrical, and abductor digiti quinti medius muscles.
Results

Silver-stained spindles

The complexity of intrafusal motor innervation is such that some endings, especially in normal spindles, had to be excluded from the analysis. Nevertheless comparison of L and PTL measurements of intrafusal motor endings from the normal and experimental muscles (see figures 3 and 4) strongly suggests that the total sample of endings studied (234, 46.2 % from normal spindles) is representative of the whole population. A sample of tracings of endings from normal and experimental muscles, selected to illustrate the range of form encountered, is shown in figure 2 and photographs of some silver-stained preparations are shown in figures 8–14, plate 1.

The results of the L and PTL measurements of the endings are illustrated by the histograms in figures 3 and 4 and are summarized in table 2. Comparisons of the populations of intrafusal motor endings on different types of muscle fibre in normal and experimental muscles are shown in table 3.

The following conclusions may be drawn.

(i) Endings on bag₁ fibres

The motor endings supplied to the bag₁ fibres of normal spindles are indistinguishable from those supplied by known β and γ axons to bag₁ fibres. However,

![Figure 4. Histograms of L and PTL measurements of cat intrafusal motor endings. These show that the measurements of endings supplied to typical chain fibres (a) and bag₂ fibres (c) in normal spindles are similar, and compare closely with those of endings supplied to these fibre types by static γ axons (b and d). Comparison of the histograms in (a) with those in figure 3d shows that endings supplied to typical chain fibres are markedly different from those supplied to long and intermediate chain fibres. The endings measured in (a) and (c) belonged to spindles in normal peroneus brevis and tenuissimus muscles; those in (b) and (d) belonged to static γ axons that had survived in the chronic degeneration experiments of Barker et al. (1973) on tenuissimus muscles.](http://rspb.royalsocietypublishing.org/)
<table>
<thead>
<tr>
<th>intrrafusal muscle-fibre type</th>
<th>L/μm</th>
<th>PTL/μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>bag&lt;sub&gt;1&lt;/sub&gt; typical chains</td>
<td>45.6, 13-107, 37</td>
<td>147.2, 15-653, 37</td>
</tr>
<tr>
<td>bag&lt;sub&gt;2&lt;/sub&gt; chains</td>
<td>71.3, 23-142, 21</td>
<td>215.1, 43-575, 21</td>
</tr>
<tr>
<td>long and intermediate</td>
<td>92.2, 25-466, 26</td>
<td>187.7, 43-576, 26</td>
</tr>
<tr>
<td>bag&lt;sub&gt;1&lt;/sub&gt; typical chains</td>
<td>36.1, 15-55, 23</td>
<td>79.7, 20-150, 23</td>
</tr>
<tr>
<td>bag&lt;sub&gt;2&lt;/sub&gt; chains</td>
<td>77.4, 35-165, 18</td>
<td>198.5, 60-474, 18</td>
</tr>
<tr>
<td>long and intermediate</td>
<td>70.2, 18-220, 58</td>
<td>149.8, 23-343, 58</td>
</tr>
<tr>
<td>bag&lt;sub&gt;1&lt;/sub&gt; typical chains</td>
<td>65.5, 21-115, 27</td>
<td>222.2, 48-355, 27</td>
</tr>
<tr>
<td>bag&lt;sub&gt;2&lt;/sub&gt; chains</td>
<td>52.0, 15-55, 23</td>
<td>215.1, 43-575, 21</td>
</tr>
<tr>
<td>long and intermediate</td>
<td>77.4, 35-165, 18</td>
<td>198.5, 60-474, 18</td>
</tr>
<tr>
<td>bag&lt;sub&gt;1&lt;/sub&gt; typical chains</td>
<td>70.2, 18-220, 58</td>
<td>149.8, 23-343, 58</td>
</tr>
<tr>
<td>bag&lt;sub&gt;2&lt;/sub&gt; chains</td>
<td>65.5, 21-115, 27</td>
<td>222.2, 48-355, 27</td>
</tr>
</tbody>
</table>

Table 2. Means, ranges, and sample sizes for L and PTL measurements of intrafusal motor endings in normal and experimental muscles.

For experimental muscles, the following ranges and means are provided:

- Static γ
  - bag<sub>1</sub> typical chains: 45.6, 13-107, 37
  - bag<sub>2</sub> chains: 71.3, 23-142, 21
  - long and intermediate: 92.2, 25-466, 26
  - bag<sub>1</sub> typical chains: 36.1, 15-55, 23
  - bag<sub>2</sub> chains: 77.4, 35-165, 18
  - long and intermediate: 70.2, 18-220, 58

- L/μm
  - bag<sub>1</sub> typical chains: 45.6, 13-107, 37
  - bag<sub>2</sub> chains: 71.3, 23-142, 21
  - long and intermediate: 92.2, 25-466, 26
  - bag<sub>1</sub> typical chains: 36.1, 15-55, 23
  - bag<sub>2</sub> chains: 77.4, 35-165, 18
  - long and intermediate: 70.2, 18-220, 58

- PTL/μm
  - bag<sub>1</sub> typical chains: 147.2, 15-653, 37
  - bag<sub>2</sub> chains: 215.1, 43-575, 21
  - long and intermediate: 187.7, 43-576, 26
  - bag<sub>1</sub> typical chains: 79.7, 20-150, 23
  - bag<sub>2</sub> chains: 198.5, 60-474, 18
  - long and intermediate: 149.8, 23-343, 58

- γ
  - bag<sub>1</sub> typical chains: 65.5, 21-115, 27
  - bag<sub>2</sub> chains: 52.0, 15-55, 23
  - long and intermediate: 77.4, 35-165, 18
  - bag<sub>1</sub> typical chains: 222.2, 48-355, 27
  - bag<sub>2</sub> chains: 215.1, 43-575, 21
  - long and intermediate: 149.8, 23-343, 58
Table 3. Comparison of populations of motor endings on different types of intramuscular muscle fibre in normal and experimental muscles

(Abbreviations: ex.m., experimental muscle; int., intermediate; n.m., normal muscle.)

<table>
<thead>
<tr>
<th>null hypothesis</th>
<th>value of $p$</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>endings A = endings B</td>
<td>(Wilcoxon’s rank sum test for 2 samples)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>endings on bag$_1$ fibres</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>all n.m. endings on bag$_1$</td>
<td>all ex.m. endings on bag$_1$</td>
<td>n.s.</td>
</tr>
<tr>
<td>$\beta$ endings on bag$_1$ (ex.m.)</td>
<td>$\gamma$ endings on bag$_1$ (ex.m.)</td>
<td>$&lt; 0.001$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>endings on chain fibres</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>n.m. endings on typical chains</td>
<td>n.m. endings on long and int. chains</td>
<td>$&lt; 0.0005$</td>
</tr>
<tr>
<td>n.m. endings on typical chains</td>
<td>n.m. endings on bag$_2$</td>
<td>n.s.</td>
</tr>
<tr>
<td>n.m. endings on long and int. chains</td>
<td>$\beta$ endings on bag$_1$ (ex.m.)</td>
<td>n.s.</td>
</tr>
<tr>
<td>n.m. endings on long and int. chains</td>
<td>$\gamma$ endings on bag$_1$ (ex.m.)</td>
<td>$&lt; 0.0001$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>static $\gamma$ endings</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>all static $\gamma$ endings (ex.m.)</td>
<td>n.m. endings on bag$_2$ and typical chains</td>
<td>n.s.</td>
</tr>
<tr>
<td>all static $\gamma$ endings (ex.m.)</td>
<td>$\beta$ endings on bag$_1$ (ex.m.)</td>
<td>$&lt; 0.0005$</td>
</tr>
<tr>
<td>all static $\gamma$ endings (ex.m.)</td>
<td>$\gamma$ endings on bag$_1$ (ex.m.)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

The endings of known $\beta$ axons on bag$_1$ fibres differ from those that remain on this fibre after the $\beta$ innervation has degenerated.

(ii) Endings on chain fibres

The motor endings supplied to typical chain fibres in normal spindles differ from those supplied to long and intermediate chain fibres, but are indistinguishable from those supplied to bag$_2$ fibres.

The motor endings supplied to long and intermediate chain fibres in normal spindles are indistinguishable from the endings supplied by known $\beta$ axons to bag$_1$ fibres.

(iii) Static $\gamma$ endings

The motor endings supplied to bag$_2$ and typical chain fibres in normal spindles are indistinguishable from the endings supplied by known static $\gamma$ axons. These endings have greater $L$ and PTL values than those of known $\beta$ axons, but differ from the endings of presumed dynamic $\gamma$ axons only with respect to their PTL values. This implies that the endings of dynamic $\gamma$ axons are more highly branched than those of static $\gamma$ axons, and axon-terminal counts confirm this (see figure 5b).

The parent axons of static $\gamma$ endings branched more frequently within the spindle than those supplying dynamic $\beta$ and $\gamma$ endings. In our samples the mean branching frequencies were 3.9 and 0.28, respectively (see figure 5a). In this respect
Figure 5. Histograms contrasting differences between static (stippled) and dynamic (unshaded) γ axons and endings. They show that, as compared with dynamic γ axons, static γ axons branch more frequently within spindles (a) and tend to supply endings that have fewer axon terminals (b) and are located nearer to the equator (c). Data in (a) and (c) from spindles in normal peroneus brevis and tenuissimus muscles; data in (b) from spindles in the experimental muscles referred to in figures 3b and 4b. p, m, d, Proximal, middle and distal thirds of polar region B.

Figure 6. Mean values of the eccentricity (a) and indentation (b) of axon terminals in 27 motor endings from cat tenuissimus spindles plotted against the distance of the endings from the primary ending. Lines drawn through the symbols are least-squares fits for regression of the ordinates on the abscissae. Note that eccentricity shows no systematic variation related to ending position, whereas indentation does. In (b) the separate origins of the data from three spindles in three different cats are indicated by the use of three different symbols.
it may be significant that the mean diameter of static γ axons in normal spindles (2.14 μm) was greater than that of dynamic axons in the same spindle sample (1.87 μm, $p < 0.01$), functional categories being inferred from the type of muscle-fibre innervated. The endings of static γ axons were located in region B, mostly in the middle third, whereas the endings of dynamic axons occurred almost exclusively in the distal third of B and in C (see figure 5c).

Figure 7. Mean values of the indentation ($I$) of axon terminals in 27 motor endings from cat tenuissimus spindles plotted against the distance of the endings from the primary ending and segregated according to their location on different muscle-fibre types. Lines drawn through the symbols are least-squares fits for regression of the ordinates on the abscissae. Circles, squares, and triangles represent the separate origins of the data from three spindles in three different cats. Endings supplied by different axons in the same spindle are indicated by different versions (filled, unfilled, half-filled) of the same symbol, so that the occurrence of two or more identical symbols indicates endings supplied by the same axon. The vertical line in each graph illustrates the range of mean values of $I$ for individual axon terminals in a single ending. Horizontal bars on this line indicate the standard error of the mean value for the whole ending.
206 R. W. Banks, D. Barker and M. J. Stacey

Table 4. Morphometric data relating to neuromuscular junctions of 27 intrafusal motor endings

<table>
<thead>
<tr>
<th>fibre type innervated</th>
<th>number</th>
<th>length/μm</th>
<th>sole-plate nuclei</th>
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<tr>
<td></td>
<td></td>
<td>range</td>
<td>mean</td>
</tr>
<tr>
<td>bag₁</td>
<td>9</td>
<td>40–106</td>
<td>64.7</td>
</tr>
<tr>
<td>bag₂</td>
<td>7</td>
<td>24–105</td>
<td>62.7</td>
</tr>
<tr>
<td>chains</td>
<td>11</td>
<td>35–59</td>
<td>45.9</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>number</th>
<th>range</th>
<th>mean per 10 μm length</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>range</td>
<td>mean</td>
</tr>
<tr>
<td>bag₁</td>
<td>2–7</td>
<td>4.9</td>
</tr>
<tr>
<td>bag₂</td>
<td>2–7</td>
<td>4.1</td>
</tr>
<tr>
<td>chains</td>
<td>1–5</td>
<td>3.0</td>
</tr>
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<table>
<thead>
<tr>
<th>mean length/μm</th>
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<tr>
<td>bag₁</td>
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<td>bag₂</td>
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<td>chains</td>
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Serially sectioned spindles

Mean values for the eccentricity (ratio E) of the axon terminals in whole endings ranged from 1.42 to 2.55 (exceptionally 3.97) and clustered around 2.0. They showed no systematic variation with respect to location of the endings (see figure 6a). Since E, I and S are interdependent, both the ratios for indentation (I) and superficiality (S) measure the degree to which terminals were embedded in the muscle-fibre surface. Hence only the results for I will be considered further.

Figure 6b shows the mean values of I for all 27 endings plotted against their distances from the primary ending. The graph clearly illustrates that the further away an ending is from the primary, the more embedded in the muscle fibre its terminals become. The results for the endings in each spindle are indicated by

Description of plate 1

Photographs of teased, silver preparations of motor endings supplied to normal cat peroneal muscles. Typical examples of the three types of intrafusal motor ending (figures 8–11, 13, 14) are illustrated together with an extrafusal motor ending (figure 12) for comparison. The ending in figure 11 is from peroneus tertius; the rest are from peroneus brevis. Scale in figure 8 applies throughout.

Figure 8. Two typical chain fibres each receive a trail plate.

Figure 9. A bag₂ fibre receives a trail plate.

Figure 10. A bag₁ fibre receives a p₁ plate.

Figure 11. A bag₁ fibre receives a p₂ plate.

Figure 12. An extrafusal motor endplate.

Figure 13. The p₁ innervation supplied to a bag₁ fibre consists of two plates 20 μm apart.

Figure 14. A long chain fibre receives a p₁ plate. Note nucleated sole plate and Doyère eminence.

These features are usually obvious in p₁ and extrafusal plates, but not in p₂ or trail plates.
Figures 8–14. For description see opposite.
Figures 15-25. For description see opposite.
different symbols; these also represent different cats since each spindle was originally located in a tenuissimus muscle of a different cat. This shows that some of the total variability arises from differences between each source of material, such as differences between individual cats, and uncontrollable variations in details of preparation.

When the results are plotted separately for the endings on each type of intrafusal muscle fibre, the mean values of $I$ increase with greater distances from the primary ending in each case. Moreover when an axon supplied two or three endings to the same muscle fibre, or the same type of muscle fibre, the fibre surface in the neuromuscular junctions of the more distal endings almost always had a greater mean indentation than that found in the junctions of the more proximal endings (see figure 7). The small number of endings sampled on bag$_2$ fibres (7), and their relatively restricted location, may account for the fact that the slope of the regression line is not significantly different from 0 in this case, though it is nevertheless similar to that in the graphs for the bag$_4$ and chain fibres. The vertical bar in each graph illustrates the variability of $I$ within a single ending (see also figures 15–25, plate 2). For example, in figure 7c the ending selected was located on a chain fibre 1.15 mm from the primary ending in region B. There were nine axon terminals in a neuromuscular junction 39 μm long; their mean length was 5.1 μm in a range of 2.0–8.0 μm. The mean value of $I$ for all terminals was $0.21 \pm 0.03$ s.e., and the mean values for individual terminals varied over the range 0.04–0.37. Finally, values of $I$ for individual terminal profiles ranged from 0 to 0.75.

The remaining data relating to the serially sectioned endings are summarized in table 4. The neuromuscular junctions on chain fibres appear to be shorter overall than those on bag fibres, but the only significant difference is that between the mean lengths of the junctions on bag$_4$ and chain fibres ($p < 0.05$, Wilcoxon’s rank sum test). The junctions on these fibre types also differ significantly ($p < 0.05$) in their number of axon terminals. This may be a consequence of the difference in junction length, but the two differences are not proportional, and the larger number of terminals on the bag$_4$ fibres may reflect a greater terminal density in their neuromuscular junctions.

### Description of Plate 2

Photographs of transverse sections through a cat tenuissimus muscle spindle showing part of a trail plate on a bag$_2$ fibre. Abbreviations: b$_2$, bag$_2$ fibre; c, chain fibre; pt., preterminal axon; s.p.n., sole-plate nucleus; S.n., Schwann-cell nucleus; t, axon terminal.

Figures 15–23. Serial, 1 μm-thick, toluidine-blue-stained sections show axon terminals of various sizes, profile, and degrees of indentation into the sole plate. The axon terminals marked by arrowheads in figure 20 are shown in detail in figure 24. The preterminal axons similarly marked in figure 21 are shown in detail in figures 24 and 25. The axon terminal labelled t in figures 19–22 is slightly more than 4 μm long. It is shown in detail in figure 25.

Figures 24 and 25. Electron micrographs of an ultrathin section taken between the sections shown in figures 20 and 21. The axon terminals are thinly covered by Schwann-cell cytoplasm which cannot be separately resolved in the light micrographs (figures 15–23). Nevertheless, the light micrographs do show fine detail; for example, the preterminal axons visible in the electron micrographs can be clearly identified in the light micrograph figure 21.
DISCUSSION

Presynaptic features

We conclude from our results that β axons are associated with a single type of intrafusal motor innervation irrespective of whether it is supplied to bag₁, long chain, or intermediate chain fibres. This type of innervation is distinctly different from the presumed dynamic γ innervation that remains on the bag₁ fibre after the β endings have degenerated following nerve section; and both these types are different from the bag₂ and typical-chain innervation, which is a single group supplied by static γ axons. In our sample of normal bag₁ innervation it is interesting to note the presence of a large peak corresponding to the β supply (see figure 3), confirming the increasing recognition of the importance of this dynamic input.

Since these three groups of intrafusal motor endings are similar to those previously identified in the literature as p₁ (β), p₂ (dynamic γ), and trail (static γ), we shall continue to use this nomenclature. We do so on the understanding that the classification is based solely on the presynaptic features of the endings, and that there is often overlap between the categories with respect to any single feature.

Postsynaptic features

Our results show that the degree of indentation of the intrafusal muscle fibres by motor axon terminals increases with greater distance from the primary ending, irrespective of fibre type. Arbuthnott et al. (1982) use degree of indentation as a major defining characteristic to classify intrafusal motor endings into various types of plate, the most deeply indented ones (their ‘mβ plates’) occurring on the bag₁ fibre. Our findings indicate that this is because the bag₁ fibre usually receives endings most distant from the primary ending (see figure 6). At equal distances from the primary there is little difference between the indentation of motor terminals on the bag₁ fibre and the other fibre types. For example, the mean value of I for the five endings in our sample that were located on bag₁ fibres 1.0–1.5 mm from the primary endings was 0.15. This compares with six bag₂ and four chain endings located within this distance whose mean values of I were 0.14 and 0.20, respectively.

Apart from the degree of indentation (primary folding) of the postsynaptic membranes, there is the amount of junctional (secondary) folding to consider. There is now good evidence that this is related to muscle-fibre type and tends to increase in motor endings on bag₁, bag₂, and chain fibres, respectively (Barker et al. 1978; Kucera 1980a, 1981, 1982b, c; Banks 1981). This gradation is matched by the cholinesterase (ChE) content of the endings, there being a parallel increase in the density and thickness of the ChE reaction product (Kucera 1982b). It may be that this reflects differences in the electrical and contractile activity of the muscle-fibre types (Pachter & Eberstein 1983). The factor of distance from the primary ending appears to operate regardless of fibre type such that the greater the distance, the higher the ChE content of an ending (Kucera 1982c), and the greater the amount of junctional folding. It follows from this, and from our own data on indentation, that maximal primary and secondary folding should be found
in motor endings supplied to long chain fibres in region C, and the observations of Arbuthnott et al. (1982, their 'm₄ plate') and Kucera & Hughes (1983a) confirm this.

Into this scheme of things Arbuthnott et al. (1982) and Sutherland et al. (1985) have introduced the idea that there are two types of static γ axon that preferentially innervate bag₂ and typical chain fibres, respectively. Both types are said to innervate chain fibres; one, mainly distributed to bag₂ fibres, supplies terminals that lie superficially on the muscle-fibre surface as 'm₄ plates', whereas the other, mainly distributed to chain fibres, supplies terminals deeply embedded in the muscle-fibre surface as 'mₑ plates'. The m₄ plates are correlated with trail endings, but the ending identity of the mₑ plates is left open. The evidence put forward by Arbuthnott et al. (1982) for the existence of the mₑ plate rests mainly on the distribution of five such plates to typical chain fibres by a static γ axon in one pole of a single tenuissimus spindle. One other such plate in another spindle is acknowledged by them to have been supplied by a dynamic γ axon, and two others in a further spindle are located on long chain fibres and were presumably supplied by a static β axon. Their study was confined to the motor innervation of six spindle poles. A study of eleven spindle poles is reported by Sutherland (1985), that claims to confirm the existence of m₄ and mₑ plates as distinct entities, but this is simply an extension of the earlier work, and, indeed, apparently includes the data from it.

We doubt whether this adds up to a valid case. In particular we doubt the histological basis for the conclusions drawn. This consisted of sampling each plate by cutting ultrathin sections at two or more levels at variable distances apart (Arbuthnott et al. 1982). In view of the variability of postsynaptic form both within and between individual intrafusal motor neuromuscular junctions, it is desirable that observations be made on serial sections.

Boyd et al. (1983) have advanced physiological evidence in support of two types of static γ axon that predominantly or exclusively innervate either bag₂ or typical chain fibres. This consists of identifying the type of muscle fibre activated by a single static γ axon in three of the spindles that it supplies. As we have shown elsewhere (Banks et al. 1985), the selection of a small number of spindles from those actually innervated by an axon inevitably tends to exaggerate any preponderant innervation of one muscle-fibre type, particularly when only two categories are recognized. This occurs even though the initial selection is unbiased. Although most static γ axons that innervate cat spindles undoubtedly show some degree of predominant distribution to bag₂ or typical chain fibres, analysis of the available histological evidence (Barker et al. 1973; Barker & Stacey 1981; Banks 1981; Kucera 1982d, 1983; Kucera & Hughes 1983b; Arbuthnott et al. 1982) indicates that this is subject to random variation.

Classification

When the cholinesterase technique was first applied to mammalian muscle spindles (Coërs & Durand 1956; Hess 1961) it became evident that, in addition to the presence of discrete plates in the poles, there were multiple diffuse endings near the equatorial region. In gold chloride preparations Boyd (1962) distinguished
these fusimotor components as ‘γ₁ plates’ and ‘γ₂ endings’, and although he illustrated several discrete γ₂ endings he chose not to describe them as plates ‘to avoid confusion with the typical end-plates of γ₁ nerve fibres’. The distinction made between ‘plates’ and ‘endings’ is thus of long standing in descriptions of mammalian intrafusal motor innervation and is expressed in the plate (p₁, p₂) and trail-ending classification of Barker et al. (1970). Indeed, the multiterminal nature of the trail ending led Barker (1967) to see it as closely resembling the grape endings that innervate the slow extrafusal muscle fibres present in some vertebrate muscles. However, the serial-section studies of Kucera (1980a, b), Banks (1981), and Arbuthnott et al. (1982) have revealed that the trail innervation is in fact distributed in the form of plates, and that in any given spindle pole the typical chain fibres, which receive most of the innervation, usually do so in the form of a single plate. We therefore propose that in future the term ‘trail ending’ should be replaced by the term ‘trail plate’.

It is clear that the postsynaptic form of intrafusal motor endings (determined by muscle-fibre type and distance from primary ending) cannot be correlated with their presynaptic form so as to produce a unified classification. The overlap in location in spindle poles is such that components of different function, or from different systems, may have the same postsynaptic form, for example, as in the case of dynamic γ and static γ endings on typical chain fibres (Arbuthnott et al. 1982), or dynamic β and γ endings on bag fibres (Banks 1981; Arbuthnott et al. 1982). There is the additional variable of fibre type, and the fact that any unified classification would have to distinguish between the static and dynamic components of both β and γ systems. We doubt whether such a classification would serve any useful purpose, though attempts to devise one have not yet been abandoned by others (Boyd & Gladden 1985; Kucera & Walro 1985).

We thank Professor J. Z. Young, F.R.S., for his comments on the manuscript, and David Hutchinson for photographic assistance.

References
Mammalian intrafusal motor endings


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