Comparison of Static Fusimotor Innervation in Cat Peroneus Tertius and Longus Muscles

FRANÇOISE EMONET-DENAND, YVES LAPORTE, AND JULIEN PETIT
Laboratoire de Neurophysiologie, Collège de France, 75231 Paris Cedex 05, France

EMONET-DENAND, FRANÇOISE, YVES LAPORTE, AND JULIEN PETIT. Comparison of static fusimotor innervation in cat peroneus tertius and longus muscles. J. Neurophysiol. 80: 249–254, 1998. Static fusimotor innervation was compared in cat peroneus longus and tertius muscles because the $\gamma$ to spindle ratio is considerably higher in the longus (~60 $\gamma$ axons for 17 spindles) than in the tertius (~24 $\gamma$ axons for 14 spindles). Single $\gamma$ axons were identified as static ($\gamma$s) by their typical effects on the response of primary ending to ramp stretch. The intrafusal muscle fibers that single $\gamma$s axons activated in the spindles they supplied were identified by the features of cross-correlograms between Ia impulses and stimuli, at 100 Hz, and by those of primary ending responses during stimulation at 30 Hz. In each experiment, a large proportion of the $\gamma$ population was tested on about nine spindles. A statistical analysis was used to estimate the number of spindles supplied by single $\gamma$s axons and the proportion of $\gamma$s axons that supply only one spindle among those the stimulation of which had activated either bag$_2$ or chain fibers alone in a single spindle. In peroneus longus, nearly all $\gamma$s axons supply one or two spindles, whereas in peroneus tertius, the majority of $\gamma$s axons supply from three to six spindles. The proportion of nonspecifically distributed $\gamma$s axons, i.e., of axons that supply both bag$_2$ fibers and chain fibers either in the same or in different spindles, is much lower (56%) in the longus than in the tertius (83%) as previously observed on a population of $\gamma$s axons that supplied from three to six spindles. Correspondingly, the proportion of specific axons is much higher in the longus (44%) than in the tertius (17%). In none of the two muscles was a strict relationship observed between the conduction velocity of $\gamma$s axons and their intrafusal distribution (specific bag$_2$, specific chain fibers, nonspecific). However, $\gamma$s supplying bag$_2$ fibers either specifically or in combination with chain fibers tended to have faster conduction velocities, which suggests that, in various motor acts, the proportion of activated bag$_2$ and chain fibers may be related to the proportions of activated fast and slow $\gamma$s axons.

INTRODUCTION

It is well established that the static fusimotor control of spindle activity is exerted through two very different types of intrafusal muscle fiber, the slow contracting nuclear bag$_2$ fibers and the fast contracting nuclear chain fibers, but uncertainty persists regarding the way static $\gamma$s axons ($\gamma$s) are distributed to these fibers, possibly because most studies on this question have been carried out on a relatively small number of axons and in few muscles (for review, see Banks 1994). Recently a quantitative analysis of the intrafusal distribution of static $\gamma$s axons was made in cat peroneus tertius muscle because, in this small muscle, it was possible to observe the actions of a large number of single $\gamma$s axons on about three-quarters of the spindle population. Using the method of Celichowski et al. (1994) for identifying the type of static effectors activated by single static $\gamma$s axons, all three possible patterns of innervation of individual spindles were observed: bag$_2$ fibers alone, chain fibers alone, and bag$_2$ and chain fibers together. In most cases, these patterns varied among the spindles supplied by each static $\gamma$ axon, showing that, in this muscle, the great majority of static $\gamma$s axons are nonspecifically distributed to bag$_2$ and chain fibers.

On average a peroneus tertius muscle contains 14 spindles (Scott and Young 1987) supplied by 24 $\gamma$s axons (Horcholle-Bossavit et al. 1988). Because about three-quarters of these axons are static, the ratio of the number of static $\gamma$s axons to the number of spindles is only slightly larger than 1 (1.2). In peroneus longus muscles, which on average contain 17 spindles (Scott and Young 1987) supplied by 60 $\gamma$s axons (Horcholle-Bossavit et al. 1988), the $\gamma$ to spindle ratio is markedly larger (2.6). This difference led us to compare the static $\gamma$ innervations in the two muscles with the aims, first, of determining whether or not peroneus longus $\gamma$s axons were all specifically distributed (some to bag$_2$, the others to chain fibers) and, second, if it was not the case and specifically and nonspecifically distributed $\gamma$s axons coexisted in peroneus longus, whether their proportions would compare with those observed in peroneus tertius. The number of spindles supplied by single static $\gamma$s axons and the conduction velocities of different sorts of $\gamma$s axons also were compared.

METHODS

Experiments were carried out on adult cats (2–2.8 kg) anesthetized with pentobarbital sodium (Nembutal, 35 mg/kg) supplemented intravenously as required. Most of the techniques used in this study have been described fully in previous papers, especially in one on the distribution of peroneus tertius static $\gamma$s axons (Celichowski et al. 1994). Static $\gamma$s axons ($\gamma$s) were identified by the typical changes their repetitive stimulation elicited in the response of primary endings to ramp stretch (Crowe and Matthews 1964; Emonet-Dénand et al. 1977). Static innervation was more difficult to study in peroneus longus than in peroneus tertius because, first, most peroneus longus $\gamma$s axons supply a much smaller number of spindles than peroneus tertius $\gamma$s axons (Brown and Butler 1975; Gioux and Petit 1993; Petit et al. 1983) and, second, because it was generally not practicable to study in each of the five experiments the actions exerted by more than one-half of the $\gamma$ population (~30 axons) on about one-half of the spindle population (9 spindles). Consequently, a statistical analysis was developed for estimating the frequencies of occurrence of $\gamma$s axons supplying different numbers of spindles (see further text).
Identification of intrafusal muscle fibers activated by \( \gamma \) s axons

This was made with the Celichowski et al. (1994)’s method, which rests on cross-correlograms between stimuli at 100 Hz and Ia afferents impulses and on the features of primary ending responses during stimulation at 30 Hz.

At 100 Hz, the contraction of the very fast contracting chain fibers is not completely fused. Therefore when chain fibers are activated in the studied spindle (either alone or with bag fibers), each periodical increase in chain fiber unfused tetanic contraction increases the probability of a Ia impulse occurring after a relatively constant delay; this results in a significant peak in cross-correlograms between stimuli and Ia impulses. When only the much slower contracting bag fibers are activated at 100 Hz, no such peaks are observed because the contraction of these fibers is completely fused.

At 30 Hz, the stimulation of chain fibers elicits either a one-to-one driving of the primary ending discharge (1 afferent impulse being elicited with a constant delay after each \( \gamma \) impulse) or a very irregular increase in the discharge of the ending with minimal instantaneous frequency that is close to that of the stimulation. On the other hand, when bag fibers are activated at that frequency, their newly fused contraction elicits a sustained and moderate increase in the instantaneous frequency. Coactivation of chain and bag fibers elicits an irregular discharge the minimal instantaneous frequency of which is well above that of the stimulation (see Fig. 2 in Celichowski et al. 1994).

Statistical analysis

The statistical analysis described below was developed by one of us (Petit) to estimate, from collected experimental data, the number of spindles supplied by individual \( \gamma \) axons and the proportions among certain axons (see Results) of \( \gamma \) s axons that supplied only one spindle.

During an experiment, among the \( N \) spindles of the muscle, a sample of \( n \) spindles (Ia fibers) was prepared. Then the numbers \( n_i \) of single \( \gamma \) axons that activated \( i \) spindles in the sample were determined; \( i \) could vary from 0 to \( i_{\text{max}} \). (It was found that \( i_{\text{max}} = 4 \) for the peroneus longus muscle and \( i_{\text{max}} = 6 \) for the peroneus tertius muscle.) The sets of numbers \( n_i \) obtained in the different experiments were compared using a nonparametric test (Kruskal-Wallis) and a parametric test (LSD, least significant differences). The sets that could be considered to belong to a single population with a 95% confidence level were pooled, giving a single set of numbers \( n_i \) for the muscle under study (1 experiment on peroneus tertius had to be discarded because in this experiment most \( \gamma \) axons activated none or only 1 spindle). The frequency that single \( \gamma \) axons activated \( i \) spindles in the observed sample was then

\[
f_i = \frac{n_i}{N_g}
\]

where \( N_g \) was the total number of \( \gamma \) axons studied in all the experiments. \( f_i \) was the frequency of ‘inactive’ \( \gamma \) axons that did not activate any spindle in the sample.

The inactive axons were observed randomly during the experiment and not particularly toward the end that would have suggested fusimotor failure. The stimulation of nearly all inactive axons was seen to increase the spontaneous afferent discharges, which were monitored continuously in the muscle nerve. As the number of Ia fibers in these thin nerves is small, the activation and/or the increase in activity of only one or two Ia afferent fibers was detected readily. In the few instances in which an increase was not obvious, it was assumed that these axons had a weak action on one or two spindles. However, it cannot be excluded that fusimotor failure, as reported by Brown et al. (1969), was responsible for the apparent ineffectiveness of some axons but the probability of such an occurrence seemed low.

Those inactive \( \gamma \) axons presumably included a small proportion of dynamic \( \gamma \) axons; this was probably very small for two reasons: the proportion of dynamic axons in our experiments was small (13% for the peroneus longus) and the probability that a dynamic \( \gamma \) axon did not activate a spindle in the sample was lower than that for static axons because dynamic \( \gamma \) axons usually supply more spindles than static \( \gamma \) axons. For these reasons, it was considered that errors due to dynamic axons were inside the confidence interval (see further).

The frequencies \( f_i \) were used to calculate confidence intervals for the probabilities \( p_{\text{ai}} \). (see Walpole et al. 1998). The probability that a \( \gamma \) axon activated \( i \) spindles in the sample was \( p_{\text{ai}} \) and the probability that a \( \gamma \) axon did not activate \( i \) spindles was \((1 - p_{\text{ai}})\). As the mean value of the frequency was \( p_{\text{ai}} \), as the standard deviation for \( p_{\text{ai}} \) was \((1 - p_{\text{ai}})\), and as \( N_g \) was large enough, we could write

\[
\text{probability } \left[ f_i - p_{\text{ai}} < \sqrt{\frac{\ln(N_g(1 - p_{\text{ai}}))}{N_g}} \right] = 1 - \frac{1}{2} \sum_{k=0}^{\infty} e^{-k^2/2} dk
\]

This probability could be calculated as a function of \( h \) and the inequality could be rewritten \((f_i - p_{\text{ai}})^2 < (h^2/N_g) p_{\text{ai}}(1 - p_{\text{ai}})\). Therefore \( p_{\text{ai}} \) had the above probability, function of \( h \), to be inside the ellipse \((f_i - p_{\text{ai}})^2 < (h^2/N_g) p_{\text{ai}}(1 - p_{\text{ai}})\).

Using this equation, for each value of \( f_i \), two values of \( p_{\text{ai}} \) could be calculated that were the upper and lower limits of the confidence interval. \( h = 2 \) for a 95% confidence level, and \( h = 1 \) for a 85% confidence level. It should be noted that for a frequency \( f_i = 0 \), the probability \( p_{\text{ai}} \) could be different from zero, \( i_{\text{max}} \) was the maximal number of spindles in the sample activated by a single \( \gamma \) axon. We assumed that the maximal number of spindles in the muscle supplied by a single \( \gamma \) axon was \( m = i_{\text{max}} + 1 \). Therefore the frequency \( f_{\text{ai}} = 0 \), but we could write the relation

\[
p_{\text{ai}} = p_{\text{ai}}^m
\]

\( p_{\text{ai}} \), probability that a \( \gamma \) axon activated \( m \) spindles in the sample. \( p_{\text{ai}} \), probability that a \( \gamma \) axon supplied \( m \) spindles in the whole muscle. \( p = n/N \) probability that a spindle was in the sample (\( p^m \) was the probability that the \( m \) spindles were in the sample).

When a \( \gamma \) axon activated \((m - 1)\) spindles in the sample, either the \( \gamma \) axon supplied \((m - 1)\) spindles in the whole muscle and the \((m - 1)\) spindles were in the sample or the \( \gamma \) axon supplied \( m \) spindles but one spindle was not in the sample. For the probabilities, the relation could be written

\[
p_{\text{ai}(\text{sample})} = p_{\text{ai}(\text{sample})} p_{\text{ai}(\text{sample})}^m + \left( \frac{m}{m - 1} \right) p_{\text{ai}(\text{sample})}^m (1 - p)
\]

\( p_{\text{ai}(\text{sample})} \), probability that a \( \gamma \) axon activated \((m - 1)\) spindles in the sample. \( p_{\text{ai}(\text{sample})} \), probability that a \( \gamma \) axon supplied \((m - 1)\) spindles in the whole muscle. \((1 - p)\) probability that a spindle was not in the sample. More generally

\[
p_{\text{ai}} = p \sum_{i=0}^{m} \left( \frac{k}{i} \right) p_{\text{ai}}^i (1 - p)^{m-i}
\]

\( p_{\text{ai}} \), probability that a \( \gamma \) axon activated \( i \) spindles in the sample. \( p_{\text{ai}} \), probability that a \( \gamma \) axon supplied \( k \) spindles in the whole muscle. \( i \) could vary from 0 to \( m \).

It was assumed that a \( \gamma \) axon supplied at least one spindle in the whole muscle. Therefore \( p_{\text{ai}} = 0 \).

\[
\left( \frac{k}{i} \right) = \frac{k!}{i! (k - i)!}
\]
RESULTS

Number of spindles innervated by single static γ axons

In peroneus longus, of 153 single γ axons prepared in five experiments, 92 were identified as static and 14 as dynamic. The action of 47 axons could not be identified because their stimulation accelerated none of the discharges of the nine Ia fibers prepared in each experiment (inactive axons).

Among the 92 static γ axons, 64 were observed to activate only one spindle of the studied sample, 24 activated two spindles, 3 activated three spindles, and 1 activated four spindles. The frequencies of occurrence of static γ axons that supplied from one to five spindles in this muscle (Fig. 1, top) were estimated by a statistical analysis (see METHODS) from the frequencies of occurrence of axons observed to activate either zero, one, two, three, or four spindles (■). In this analysis on 139 γ axons (92 γs + 47 inactive axons), the inactive axons were treated as if they were all static for two reasons: the proportion of identified dynamic γ axons observed in this study was low (13%) and the probability of finding inactive dynamic axons is lower than that of finding inactive static axons because dynamic γ axons generally supply a larger number of spindles than most static axons. The errors resulting from that simplification were considered as being within the confidence intervals (see METHODS).

In peroneus tertius of 90 single γ axons prepared in six experiments (from Celichowski et al. 1994), 59 were identified as static, 22 as dynamic, and only 9 were inactive. Among the 59 static axons, 15 were observed to activate one spindle, 9 activated two spindles, 19 activated three spindles, 9 activated four spindles, 6 activated five spindles, and 1 activated six spindles. The estimated frequencies of occurrence of single static γ axons supplying from one to seven spindles are indicated in Fig. 1, bottom. In marked contrast with peroneus longus, in which nearly all γ static axons supply either one or two spindles, the majority of peroneus tertius static γ axons supply from three to five spindles but there is also a noticeable proportion of axons that supply only one spindle.

Intramuscular distribution of static γ axons

The distribution of single γ axons to intramuscular muscle fibers (bag2 and chain fibers) was determined in each of the spindles they activated. All three possible patterns of innervation were observed in individual spindles: bag2 fiber alone, chain fibers alone, and bag2 and chain fibers together. In this last case, there were large variations in the proportions of the actions exerted by the two types of fibers as previously reported (Celichowski et al. 1994).

In peroneus longus, among the 64 γs axons that were observed to activate only one spindle in the examined sample, 46 supplied only one type of intramuscular muscle fiber, either chain fibers (37) or bag2 fibers (9). Calculated probabilities (see METHODS) indicated that, of these 46 axons, 22 axons did supply only one spindle in the muscle, whereas the 24 others supplied more than one spindle. Therefore 22 axons were maintained in the group of axons that could be classified because of their known distribution in that single spindle, whereas 24 axons were not considered further because there was no way of determining their complete distribution.

The original group of 92 γs axons thus was reduced to 68. Of these 68 axons, 38 (56%) were classified as nonspecifically distributed and 30 (44%) as specifically distributed. Among these axons, eight were observed to activate the same type of intramuscular muscle fibers in two spindles (bag2, in 2 instances, chain fibers in 6 instances) and were classified as specifically distributed because, in peroneus longus, γ axons that supply more than two spindles are extremely rare. The 22 other axons were classified as specifically distributed, either to bag2 fibers (4 axons) or to chain fibers (18 axons) because either type of fibers alone were activated in the single spindles these axons were estimated to supply.

Thus in peroneus longus the proportions of nonspecific
In Fig. 3, the conduction velocities of 12 peroneus tertius \( \gamma \) axons that supplied bag\(_2\) fibers in at least three spindles either alone or with chain fibers (2 and 10, respectively) are compared with those of 30 other axons that include specific chain axons and nonspecific axons with occasional bag\(_2\) fibers.

The conduction velocities of the axons of the first group ranged from 29 to 44 m/s (mean conduction velocity 35 \( \pm \) 4.4 SD), whereas those of the axons of the second group ranged from 22 to 40 m/s (mean conduction velocity: 29 \( \pm \) 4.4 SD). The variances for the two groups are not significantly different (\( F \) test), but the two means are significantly different (\( t \)-test, 95% confidence level).

In peroneus longus, when all \( \gamma \) axons are considered together, this tendency is blurred because of large individual variations in the conduction velocity ranges of these axons and in the numbers of active axons prepared in each experiment. Moreover, although the statistical analysis (see preceding text) allowed the inclusion in the group of classifiable axons of nearly one-half of the axons that activated either bag\(_2\) or chain fibers alone in a single spindle, they could not be individually identified; therefore no conduction velocities could be ascribed to these axons.

For these reasons, conduction velocities of all active \( \gamma \) axons observed in each of the five experiments are presented in the histograms of Fig. 4. It can be seen that, in spite of the individual differences, axons that supply bag\(_2\) fibers either alone (\( \square \)) or with chain fibers (\( \square \)) tend in each experiment to be faster than those that supply only chain fibers (\( \blacksquare \)).

**DISCUSSION**

The intrafusal distribution of static \( \gamma \) axons was compared in cat peroneus longus and tertius muscles because there are almost three times as many \( \gamma \) axons in the longus than in the tertius although the spindle contents of the two muscles are comparable. Large differences were observed. First, nearly all static \( \gamma \) axons in peroneus longus supply one or more chain fibers (Table 2). The number of axons that supplied bag\(_2\) fibers only was, however, much smaller in the longus than in the tertius (Table 2). In the longus, bag\(_2\) axons were less numerous, especially in the slow-conducting group, where the frequency of bag\(_2\) axons was nearly zero (Fig. 4, far right). Conversely, in the tertius, axons that supplied bag\(_2\) fibers only were more frequent in the fast-conducting group (Fig. 4, far right).

In the longus,bag\(_2\) fibers were spread mainly among the slowest axons (Fig. 4, far right), and in the tertius, bag\(_2\) fibers were more dispersed among the fast-conducting axons (Fig. 4, far right). Axons that supplied bag\(_2\) fibers only were much more frequent in the fast-conducting group in the tertius than in the longus (Table 2). In the longus, bag\(_2\) fibers were spread mainly among the slowest axons (Fig. 4, far right), and in the tertius, bag\(_2\) fibers were more dispersed among the fast-conducting axons (Fig. 4, far right). Axons that supplied bag\(_2\) fibers only were much more frequent in the fast-conducting group in the tertius than in the longus (Table 2).
The much larger $\gamma$ to spindles ratio found in the longus does not allow by itself to predict the distribution of $\gamma$ axons because, theoretically, static $\gamma$ axons, whatever their number, could all be distributed either specifically or nonspecifically. However, the fact that the proportion of specifically distributed axons is much higher in the longus than in the tertius raises the possibility that the degree of specificity is determined probabilistically, at least in part. The $\gamma$ to spindles ratios in longus and tertius are among the largest and the smallest, respectively, of all such ratios so far measured (Boyd and Davey 1968). If in several muscles with ratios falling between these extreme values, the proportions of specifically distributed axons consistently were found to be related to the ratios, the probabilistic nature of the $\gamma$ distribution then would be strongly supported. However, it is likely that other factors contribute to the distribution. This is suggested, for instance, by the slight tendency toward segregated innervation of $\beta_2$ and chain fibers that was previously observed in individual spindles in the tertius (Celichowski et al. 1994) and that also was found in the longus (unpublished observations). This segregation is not in itself an evidence for the existence of specifically distributed axons because, in the tertius for example, most static $\gamma$ axons were observed to be distributed randomly to one or other fiber types or both among the several spindles (from 3 to 6) individual axons supplied (Celichowski et al. 1994).

The flexion-abduction of the foot elicited by the longus would seem to necessitate a more precise control than the extension-abduction of the fifth digit elicited by the tertius. The features of the static innervation of the longus spindles should contribute to this finer control because a large number of static $\gamma$ axons and a high proportion of specifically distributed axons suggest a finer control of the sensory endings discharges than in tertius spindles. However, other factors should be considered such as the $\beta$ innervation, which is particularly developed in tertius spindles (Jami et al. 1982) and perhaps could compensate for the comparatively low number of $\gamma$ axons.

No strict relationship was found between the conduction velocities of axons and their distribution to $\beta_2$ fibers only, chain fibers only, or $\beta_2$ and chain fibers together. However, in both, muscles $\gamma$ axons that supplied $\beta_2$ fibers, either alone or in combination with chain fibers, tended to have faster conduction velocities (i.e., a larger axonal diameter) than other axons, in agreement with previous reports (Banks 1991; Emonet-Dénand and Gladden 1993) and with observations in tibialis posterior showing that slow $\gamma$ axons preferentially supply chain fibers (Brown et al. 1965). If such a tendency exists in all muscles, the proportion of $\beta_2$ and chain fibers activated in various motor acts might be determined by the proportion of activated fast and slow $\gamma$ axons, whether the recruitment of their respective motoneurons is related or not to the size of these neurons.

The proportion of activated $\beta_2$ and chain fibers of course can be modified by the recruitment in various proportions of specific $\gamma$ motoneurons, but it should not be overlooked that chain action also could be substantially enhanced by a large increase, even temporary, in the frequency of discharge of the many $\gamma$ motoneurons that supply both $\beta_2$ and chain fibers. The contraction of the relatively slow $\beta_2$ fibers will not increase beyond 50–60 Hz, whereas that of the much

![Fig. 4. Conduction velocities of peroneus longus static $\gamma$ axons observed in 5 experiments (A–E). Classes of 2.5 m/s. A, C, and E, axons that were observed to activate either chain fibers alone, $\beta_2$ fibers alone or chain, and $\beta_2$ fibers together respectively, either in only 1 of the sampled spindles (boxes with no dots) or in $>1$ spindle (boxes with a dot).](image-url)
faster twitch chain fibers will continue to increase up to frequencies as high as 150–180 Hz.

The complementarity of the actions exerted by bag and chain fibers recently was shown in peroneus tertius spindles during sinusoidal muscle stretch of linearly increasing frequency (Emonet-Dénand et al. 1997), but most of the functional consequences of this coactivation remain to be elucidated.

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Address reprint requests to Y. Laporte.

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