Characterization of Spindle Afferents in Rat Soleus Muscle Using Ramp-and-Hold and Sinusoidal Stretches

LAURENT DE-DONCKER,1 FLORENCE PICQUET,1 JULIEN PETIT,2 AND MAURICE FALEMPIN1
1Laboratoire de Plasticité Neuromusculaire, EA 1032, IFR 118, Bât. SN4, Université des Sciences et Technologies de Lille 1, F-59655 Villeneuve d’Ascq Cedex; and 2Faculté des Sciences du Sport et de l’Éducation Physique, Université Bordeaux 2, Domaine Universitaire, F-33607 Pessac Cedex, France

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INTRODUCTION

The postural and locomotion controls are complex and require different proprioceptive and exteroceptive receptors. In the literature, among all these receptors, the muscle spindle plays a fundamental role (McCloskey 1978; Prosek et al. 2000). This stretch receptor is inserted in parallel with the extrafusal fibers (for review, see: Hunt 1990; Prosek 1997). Each muscle spindle consists of a hyaluronic acid fluid-filled capsule. Inside, there is a small number of modified muscle fibers, called intrafusal fibers, each having two contractile ends, the poles, and an equatorial region almost devoid of myofibrils and containing myonuclei (Banks et al. 1982; Hulliger 1984). Two main types of intrafusal fibers are identified: the nuclear chains and the nuclear bag fibers (bag1 and bag2) differing in the way in which the nuclei are distributed, their diameters and cross sectional areas, and their immunohistochemical properties. Two kinds of endings innervate the muscle spindle: the primary and the secondary endings are supplied in the cat by Ia and II fibers, respectively. These fibers exhibit different responses to imposed ramp-and-hold stretch (Cheny and Preston 1976a,b; Crowe and Matthews 1964; Jam and Petit 1979; Matthews 1963). The discharge of Ia fibers indicate both the muscular length changes (static sensitivity) and the velocity of length changes (dynamic sensitivity), whereas the discharge of II fibers provide mainly information about the length changes (McCloskey 1978). The sensitivity of primary and secondary endings to dynamic and static changes in muscle length are controlled by two types of motor neurons from the CNS: the fusimotor neurons (γ neurons) and the m fusimotor neurons (β neurons) (Banks 1994; Hunt 1990; Petit et al. 1999; Prosek 1997).

The literature reporting on morphological (for review, see Arbuthnot et al. 1989; Hulliger 1984; Maier 1997), histochemical, and immunohistochemical (Pedrosa-Domellöf et al. 1991; Soukup et al. 1995) properties of rat muscle spindles is quite profuse. The data obtained in rat are in agreement with those obtained in the cat. However, although the discharge characteristics of the Ia and II fibers have been extensively studied in cat (Hunt 1990; Matthews 1972) and primate (Cheney and Preston 1976a,b), very few data are available on the discharge of rat muscle spindle afferents (Andrew et al. 1973; Hnik et al. 1977; Miwa et al. 1995). This is highly regrettable, because the rat model has been extensively used in studies based on neuromuscular disuse or training, the characteristics of rat spindle afferents being, however, less substantiated than in other spe-
cies. Therefore the aim of our study was to determine unequivocal criteria to classify the afferent responses from passive soleus muscle spindle (i.e., without fusimotor outflow) of control rats into primary and secondary endings responses. To carry out the present work, the discharge characteristics of rat muscle spindle afferents were analyzed with ramp-and-hold stretches at different velocities and amplitudes and with sinusoidal stretches at different amplitudes and frequencies. Several parameters already defined and measured in cat (Hulliger et al. 1976; Hunt 1990; Hunt and Ottoson 1975; Hunt et al. 1978; Matthews and Stein 1969) and primate (Cheney and Preston 1976a,b) were used. However, in the literature, no criteria allow to clarify unambiguously the discharges of Ia and II fibers, and several parameters are often used to perform this classification. Thus the aim of this work was to determine criteria allowing to classify easily and unequivocally the discharges of the Ia and II fibers of rat soleus muscle spindles.

M E T H O D S

Animals

Experiments were performed on 15 male Wistar rats (IFFA CREDO, L’Arbresle, France) weighing 280–300 g. All the rats were housed individually in separate cages. They were maintained at a temperature of 25 ± 1°C with a 12/12 h circadian cycle. All the experiments as well as the maintenance conditions of the animals received authorizations from both the Agricultural and Forest Ministry and National Education Ministry (Veterinary Service of Health and Animal Protection: authorization 59-00980).

Surgical technique

Each rat was anesthetized with intraperitoneal injection of pentobarbital sodium (30 mg/kg). Supplementary injections (15 mg/kg) were provided when necessary. At the end of the experiment, the animals were killed with a lethal dose of anesthetic (100 mg/kg). Under deep anesthesia, assessed by the absence of blink reflexes, all the muscles of the thigh and lower right hindlimb were denervated except the soleus muscle. The soleus blood supply was kept intact. Under a stereomicroscope, the soleus nerve was freed and cleaned. In situ, the minimal (L_{min}) muscle length (3 ± 0.08 cm, measured at full ankle extension) and the maximal physiologic (L_{max}) muscle length (4.1 ± 0.02 cm, measured at full ankle flexion) were determined.

A laminectomy was performed between L_{3} and L_{6}. A mineral oil pool was achieved with the dorsal skin of the rat around the incision and with a pause in their discharge during the twitch or a brief tetanic contraction. Several parameters were used: the presence of a phase lead decrease at the peak of the twitch muscle. The axonal conduction velocities were calculated as the ratio of the nerve conduction distance to the antidromic spike delay. The conduction distance was measured after postmortem dissection. Muscle spindle afferent spikes were recorded using a digital tape recorder (DTR 1404, Biologic Science Instruments, Clai, France), and a CED 1401 interface with the Spike 2 processing package (Cambridge Electronic Design, Cambridge, UK), which converted the analogic discharge to an instantaneous discharge frequency.

Parameters used to identify the muscle spindle afferents

RAMP-AND-HOLD STRETCH. Ramp-and-hold stretches were applied at three different initial muscle lengths: L_{min} + 10%L_{max} and L_{min} + 20%L_{max} (110, 115, and 120% of L_{min}, respectively). These lengths were set using a micrometer and were included in the physiological range between the L_{min} and the L_{max} muscle lengths. For each length, after a prestretch of 1-mm ramp-and-hold stretches were applied with amplitude ranges of 3 mm (S3) and 4 mm (S4) at 6, 10, 15, and 30 mm/s velocities. The plateau phase was held for 5 s. Two stretches were separated by 25 s. Each series of stretches was repeated five times with the same parameters.

Several parameters were measured to characterize primary and secondary responses: the value of the resting discharge (RD) during the 0.5 s before the start of the stretch, the dynamic peak discharge (DP) that was the value of the discharge frequency at the end of the ramp phase, the static final value (FST) that was the mean value of the discharge frequency at the end of the 5-s plateau phase, the dynamic index (DI) that was the difference between the DP and the frequency at 0.5 s after completion of the stretch (Crowe and Matthews 1964; Hunt et al. 1963), the presence or the absence of a discharge during the stretch release was also studied (Hunt 1990; Hunt and Ottoson 1975; Hunt et al. 1978), and the static sensitivity that was the difference between the static response (FST - RD) divided by the amplitude of the stretch (Boyd 1981). The significance of RD, DP, DI, and FST parameters is illustrated in Fig. 1.

SINUSOIDAL STRETCH. Sinusoidal stretches (0.5-, 1.0-, 2.0-, 6.0-, and 10-Hz frequencies) and vibrations (50-, 100-, and 150-Hz frequencies) were applied at 0.12, 0.25, 0.5, and 1 mm/s stretch amplitudes defined by 10.220.32.247 on August 28, 2017 http://jn.physiology.org/ Downloaded from
Statistical analysis

The linear regression slopes of DI as a function of the stretch velocities were determined for each muscle spindle fiber. From these slope values, a distribution histogram was achieved using GraphPad Prism 3 software to determine the presence of different fiber populations. The significant differences of results expressed as means $\pm$ SE were determined by using a nonpaired Student's $t$-test ($P < 0.05$).

RESULTS

The results are based on recordings from 51 spindle soleus afferent units of rat.

The RD, FST, static sensitivity parameters measured under a ramp-and-hold stretch, and response amplitude, sensitivity, and phase lead parameters obtained after a sinusoidal stretch did not permit to immediately distinguish two fiber populations. Therefore except for the conduction velocities, only the parameters that allowed to visually identify two kinds of fibers were retained.

Afferent discharges during ramp-and-hold stretches

Under ramp-and-hold-stretch of 3-mm amplitude applied at $L_{\text{min}}+20\%$, and at 3-mm/s velocity, two types of responses were observed and are illustrated in Fig. 1. Both groups of responses showed a RD before the beginning of the stretch and a sustained discharge which determined the FST at the end of that phase. One type of response exhibited a high dynamic peak (125 $\pm$ 6.7 Hz) and a pause in the discharge during the stretch release. The other type of response was characterized by a lower DP value (63 $\pm$ 3.5 Hz) and a continuous discharge during the stretch release. Twenty-six responses belonged to the first group and 25 to the second group. Four responses with intermediate properties could not be classified and were not included in the sample.

Conduction velocity of afferent fibers

Figure 2 presents the histogram of the conduction velocities for 51 muscle spindle afferents. This figure does not show a bimodal distribution. However, the asymmetry of the histogram suggests the existence of two peaks, the first one $\sim 32-36$ m/s and the second $\sim 40-44$ m/s.

Dynamic index

The relations between the DI and the stretch velocity were studied at $L_{\text{min}}+10\%$. The linear regression slopes of these relations were determined from the discharges of 51 spindle afferent fibers at $L_{\text{min}}+10\%$ and for the two stretch amplitudes. The distribution histogram of these slopes (Fig. 3) shows a bimodal distribution. With a 3-mm amplitude stretch, the first peak was at 0.8 and the second at 4.3. With a 4-mm amplitude stretch, these peaks were shifted toward higher values, 1.3 and 5.3, respectively. Twenty-five fibers were in the part of the distribution around the first peak, and 26 fibers were in the part of the distribution around the second. Similar bimodal distributions were observed in the histograms of the DI parameter for 3- and 4-mm amplitude stretches at $L_{\text{min}}+15\%$ and $L_{\text{min}}+20\%$ (not illustrated). When the muscle length increased, the peak with the highest slope value was shifted toward lower slope values, whereas the first peak had the same slope value. At $L_{\text{min}}+20\%$, the second peak had a slope of 3.3 at 3-mm amplitude and 4.8 at 4 mm.

For the three initial muscle lengths and for each stretch amplitude, we could divide the responses into two groups based on slope, and we could verify that, each time, the two groups of responses corresponded to the same two groups of afferent fibers. Our data also showed that the increase in DI values in both fiber groups linked to the increase in stretch...
velocity was more pronounced when the initial muscle length was maintained at low length ($L_{\text{min} + 10\%}$). For a constant stretch amplitude, the mean DI values of the first group of fibers increased depending on the initial muscle length (between $L_{\text{min} + 10\%}$ and $L_{\text{min} + 20\%}$), whereas this increase was less marked for the second group of fibers. The dynamic responsiveness of the first group fibers thus became more pronounced when the stretch amplitude was large. It is important to say that the group of fibers with higher slope values presented a higher dynamic peak and a pause in the discharge during the stretch release. The group of fibers with lower slope values had a small dynamic peak and a continuous discharge during the stretch release. We assumed that the group of afferent fibers that corresponded to the highest slope values innervated primary endings and that the second group innervated secondary endings.

It should be noted that for each initial length and for each stretch amplitude and stretch velocity the mean DI values of the first group were four times as high ($P < 0.05$) as the mean DI values of the second group. The overlap in the conduction velocity values was in the 36- to 40-m/s interval. Although an overlap appears in the conduction velocity values of afferent fibers (Fig. 2), the mean conduction velocity of the first fiber group ($43.3 \pm 0.8$ m/s) was significantly higher than that of the second fiber group ($33.9 \pm 0.9$ m/s).

Discharge of muscle spindle afferents during sinusoidal stretches

Experiments were performed on 33 endings at $L_{\text{min} + 20\%}$ under sinusoidal stretches and vibration stimuli. These endings were part of the 51 endings studied previously under ramp-and-hold stretch.

Linear range

As expected, two types of responses, (Fig. 4), were observed. Fifteen and 18 fibers belonged, respectively, to the first and second groups. The first and second fiber groups obtained under sinusoidal stretches corresponded to the first and second fiber groups previously described after ramp-and-hold stretches. Figure 4 shows the two types of responses to 0.5-Hz stretches with amplitudes ranging from 0.12 to 1 mm. The linear range of the 15 fibers of the first group extended from 0.12 mm (between 0.5 and 3 Hz) to 0.25-mm stretch amplitude (between 0.5 and 2 Hz). Beyond 0.25-mm amplitude, the discharge became discontinuous. The linear range of the 18 fibers of the second group extended from 0.12 to 1 mm of stretch amplitude between 0.5- and 3-Hz stretch frequencies.

Responses to vibrations

Vibrations were applied at $L_{\text{min} + 20\%}$ to the distal tendon of the soleus muscle. Their amplitudes were within the respective linear range of the first and second fiber groups and their frequencies were of 50, 100 and 150 Hz. The results are reported in Table 1.

At 50 Hz and with a 0.12-mm stretch amplitude, the discharge of the 15 fibers of the first group were 1:1 driven (1 imp/sinusoidal cycle) by the vibration. At 100 Hz and for the same stretch amplitude, 12 fiber responses featured 1:1 driving and 3 fiber responses featured 1:2 driving. At 150 Hz and for the same stretch amplitude, 10 fiber responses featured 1:1 driving and 5 fiber responses featured a 1:2 driving. When the amplitude of vibrations was increased to 0.25 mm, the fiber responses of the first group were all driven by the vibration at the three vibration frequencies.

For the 18 fibers of the second group, driving was rarely observed with vibration amplitude $\leq 0.25$ mm (Table 1). With a 0.5-mm vibration amplitude, 1:1 driving was the response of 14 fibers to 50-Hz vibration, 10 fibers to 100-Hz vibration, and 7 fibers to 150-Hz vibration (see Table 1 for details of non-driven afferent discharges). The discharge of every fiber of the second group was 1:1 driven by a 50-Hz vibration with a 1-mm amplitude. Almost all the fibers of this group had the same kind of response to 100- and 150-Hz vibrations (Table 1).

Discussion

The aim of our study was to differentiate discharges coming from Ia and II afferent fibers. We could have distinguished Ia fibers lacking a bag1 fiber from IIa fibers innervating this intrafusal fiber by using succinylcholine. However, several authors have shown that the primary endings exclusively innervating bag2 and chain fibers displayed a similar dynamic range.
response (under passive condition) to that of primary afferents innervating all intrafusal fiber types (Gioux et al. 1991; Scott 1991).

In the present investigation, we studied the characteristics of the discharges of rat soleus muscle spindles and we measured the discharge parameters commonly used in other species (cat, primate, human). Our results demonstrated that in rat, contrary to what had previously been reported in cat, the histogram of

![Image](https://example.com/image.png)
TABLE 1. Vibration sensitivity of the two fiber groups

<table>
<thead>
<tr>
<th></th>
<th>First Fiber Group</th>
<th>Second Fiber Group</th>
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<tbody>
<tr>
<td></td>
<td>0.12 mm 0.25 mm</td>
<td>0.12 mm 0.25 mm 0.5 mm 1 mm</td>
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<tr>
<td><strong>Amplitudes</strong></td>
<td></td>
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<tr>
<td>50 Hz</td>
<td>50 Hz (15)</td>
<td>25 → 50 Hz (18)</td>
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<td>100 Hz</td>
<td>12: 100 Hz (15)</td>
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<td>150 Hz</td>
<td>10: 150 Hz (15)</td>
<td>10: 150 Hz (18)</td>
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<tr>
<td><strong>Vibration frequencies</strong></td>
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<tr>
<td>50 Hz</td>
<td>150 Hz (18)</td>
<td>4: 25 Hz (18)</td>
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<tr>
<td>100 Hz</td>
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<td>10: 100 Hz (18)</td>
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<td>150 Hz</td>
<td>150 Hz (18)</td>
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Discharge frequency of the first group (n = 15; in parentheses) and second group of fibers (n = 18) under vibration stimuli applied at different frequencies and amplitudes. 25 → 50 Hz indicates that the discharge varied from 25 to 50 Hz.

conduction velocities was not clearly bimodal (Boyd and Davey 1968; Wei et al. 1986); it was thus hazardous to assume that all primary endings were innervated by Ia fibers and all secondary endings by II fibers. Using functional criteria to discriminate primary endings from secondary endings, we were able to show that an overlap in the conduction velocities of the afferent fibers innervating these endings exists. Such an overlap also exists in cat but is less important than in rat. Boyd (1962) and Banks et al. (1982) have shown in cat that different types of secondary endings were distinguished following their position on either side of the primary ending innervation. The S1 endings lay immediately adjacent to the primary endings and were formed by the largest-diameter II axons. The majority of these axons innervated all three fiber types. The S2 and S3 endings lay further from the primary endings, and progressively had smaller axons. The S2 ending fibers innervated mainly bag2 and/or the chain fibers, whereas the S3 endings lay predominantly on the chain fibers. Banks et al. (1982) have also shown that the diameter of the Ia axons supplying bag2 and chain fibers was generally smaller than that of the axons supplying all the intrafusal fibers. Therefore these data could explain the overlapping of fiber conduction velocities described in the rat.

Ramp-and-hold stretch

In our study, the RD and the FST parameters did not permit to immediately distinguish two fiber populations. They were therefore discarded. Our data showed that in the absence of fusimotor activity, a first fiber group stopped firing abruptly during the release of a small (3 mm) and slow (3 mm/s) stretch, whereas a second fiber group did not cease to fire. The distribution histogram of the DI linear regression slopes was clearly bimodal without overlapping, and the DI values in the first group fibers were greater than those of the second group fibers. This difference was found at all muscle lengths, velocities and stretch amplitudes. Moreover, at a given muscle length, DI values increased both with velocity and stretch amplitude in both fiber groups. However, this was less marked and variable for the second group of fibers. It has early been demonstrated during the release of a small and slow ramp-and-hold stretch that in cat, the Ia fibers ceased to fire, whereas II fibers continued (Hunt 1990; Hunt and Ottoson 1975; Hunt et al. 1973). In cat (Matthews 1963; Wei et al. 1986), primate (Cheney and Preston 1976a,b), and human (Edin and Vallbo 1990), the Ia and II fibers from hindlimb soleus muscle are characterized by well-separated ranges of DI, which permit differentiation of the fibers. The DI values of Ia fibers increased with the stretch velocity (Holm et al. 1981; Houk et al. 1981; Matthews 1963), stretch amplitude (Fisher and Schäfer 2000; Matthews 1972), and muscle length (Houk et al. 1981). On the contrary, it has been demonstrated that the DI of Ia fibers was often independent of the initial muscle length (Cheney and Preston 1976b; Matthews 1963). Furthermore, Cheney and Preston (1976b) have also observed that when the initial length was kept constant and the stretch amplitude varied, the DI of Ia fibers was often greater for low-amplitude stretches.

From our data and according to those previously described in the literature, it was tempting to suggest that our first group fibers corresponded to Ia fibers and our second group afferents to II fibers.

The differences in dynamic response between Ia and II fibers could be due to the location of Ia and II afferent fibers along the intrafusal fibers (Banks et al. 1982; Boyd 1962; Cheney and Preston 1976b) and the distinct mechanical properties of intrafusal muscle fibers innervated by Ia and II fibers (Andrew et al. 1973; Boyd et al. 1977; Hulliger 1984; Corvaja 1969; Matthews 1972; Poppele and Quick 1985; Scott 1990).

Although there was a clear difference between the DI values of the first and second group fibers, other parameters were used to confirm this classification.

Linear range of both fiber groups during a sinusoidal stretch

As the response amplitude and the phase lead did not make it possible to immediately identify two fiber populations, we only retained the parameters that allowed us to visually identify two kinds of fibers.

In our study, the amplitude of the response in both fiber groups increased linearly with the amplitude of the sinusoidal stretch over a limited range. This linear range extended from 0.12 to 0.25 mm for the first group fibers and from 0.12 to 1 mm for the second group fibers. The linear range of the second group fibers was therefore broader than that of the first group afferents at all stretch frequencies. Beyond 0.25 mm, the estimation of the response amplitude was very uncertain because the first group fibers failed to evoke action potentials during the
whole sinusoidal cycle. Similarly, in cat (Hasan and Houk 1975; Hulliger et al. 1977; Matthews and Stein 1969) and in human (Kakuda 2000), II fibers presented a broader linear range than Ia fibers. Moreover, Laporte and Emonet-Dénard (1973) have shown in cat that sinusoidal stretches <0.5 mm amplitude were sufficient to elicit bursts of impulses in Ia fibers, whereas the discharge of the II fibers was sinusoidally modulated. Therefore in accordance with these results, our data constitute another argument confirming that the first and second group fibers belong to Ia and II fibers, respectively.

Vibrations

Vibrations were applied to the distal tendon of the soleus muscle at \( L_{\text{min}} + 20\% \). This length was chosen to keep the muscle and muscle spindles under tension and thus to get a better sensitivity to vibration. Indeed, the ability of spindles to get slack is known to decrease progressively at longer muscle lengths as passive tension increases (Gregory et al. 1986). Our results showed that for the three vibration frequencies (50, 100, and 150 Hz) used, the amplitude threshold of the second group fibers was higher than that of the first group fibers to produce one spike per cycle of a sinusoidal stretch. For example, at 0.12-mm stretch amplitude, almost all the first group fibers discharged at 100 Hz for vibrations applied at 100-Hz frequency, whereas the majority of the second group fibers discharged at this frequency for 1-mm stretch amplitude. Therefore to get the same discharge frequency, the thresholds of vibration amplitude were higher for the second group fibers than for the first. To confirm our data, it has been observed that high-frequency vibrations of small amplitude (0.1 mm) constituted specific stimuli for Ia fibers (Brown et al. 1967; Matthews and Watton 1981; Prosek et al. 2000; Roll et al. 1989), which led these fibers to discharge to one spike per sinusoidal cycle.

To conclude, our study demonstrated that in rat soleus muscle spindles, it was possible to immediately distinguish two groups of fibers by using some significant parameters during ramp-and-hold (DI, discharge during stretch release) and sinusoidal stretches (linear range, vibration sensitivity). In comparison with data obtained by several authors, all these parameters used in combination showed that the first group fibers corresponded to Ia fibers, whereas the second group fibers were classified as II fibers.

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