Impulse patterns of single encoding sites of human secondary muscle spindle afferents

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Abstract

1. *By recording single-fibre action potentials (APs) from human sacral nerve roots, the impulse patterns of 6 secondary spindle afferents could be identified, analysed and compared with the impulse trains of oscillatory firing α_2-motoneurons.*
2. *Secondary spindle afferents fired with single APs, doublets, bursts and equidistant APs. On the assumption that the most simple firing pattern of a single fibre with a shortest interspike interval (II) of 80 msec was generated by a single encoding site, the firing patterns of the other afferents could be splitted into firing patterns of single encoding sites with similar II distributions and also with a shortest II of about 80 msec.*
3. *Successive IIs also reflected the different ways of firing of the secondary muscle spindle afferents. A fibre from a paraplegic with bladder dyssynergia seemed to show a higher activity than a comparable probably normal fibre. The doublet IIs of two secondary spindle afferents ranged from about 9 to 14 msec with peaks at 10.2, 11.2 and 13 msec in the distributions. Multiple encoder sites increased discharge rates of parent fibres, but did not always regularize the output discharge.*
4. *The simultaneously plotted impulse patterns of 4 secondary spindle afferents showed coordinated firing: they did not fire simultaneously but regularized the summed activity. A phase of 50 msec (range 30 to 70 msec) often occurred between the spindle afferent APs and the impulse trains of an oscillatory firing α_2-motoneuron. An afferent drive of the oscillator with a certain phase is likely.*

Key-words: Human — Secondary spindle afferents — Encoder sites — Shortest interspike interval — Doublet firing — Burst firing — Spindle coordination.

Introduction

In previous papers the oscillatory firing of motoneurons were analysed which activate the sphincters for the continence of the rectum (14, 16) and the urinary bladder (14, 17). However, an oscillatory system only oscillates if supplied with energy in phase. A pendulum needs a certain push in phase to swing, because of the existing damping. An electronic oscillator needs recoupling with a certain phase to oscillate. The oscillatory systems under consideration here are the sphincteric motoneurons with sets of interneurons (16), which start to oscillate in brain-dead humans (HTs) or paraplegics, if certain afferent input in phase activates and drives the oscillatory systems. Since at least some motoneurons innervating the external anal sphincter fire in the oscillatory firing mode, because of the constant stretch reflex of the anal sphincter (14, 19), the afferent drive comes mainly from the stretched muscle spindle. The secondary muscle spindle afferents (only very few primaries were found till now (13)) should have a suitable pattern to drive the oscillatory

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system (14). The afferent impulses contributing to the oscillation must also have a rather constant phase to the motoneuron impulse trains. The afferent impulsation pattern can originate from the spindle itself (stretch) or be formed in cooperation with the activity of the efferent spindle innervation. Synchronicity of the spindle afferent action potentials (APs) with the motoneuron impulse trains however, can only come from the activity of the efferent spindle innervation. A further step be clarified is, where the efferent spindle innervation, consisting mainly of γ-motoneurons (intrafusal), gets their fixed phase drive from. Since secondary spindle afferents possess several myelinated endings (1), multiple sources of APs can be expected (1, 7, 8, 11). A thorough analysis of possible phase relations between the afferent and efferent spindle APs requires the knowledge of the impulse patterns of single afferent encoder sites. In this paper mainly impulse patterns of secondary spindle afferents are analysed, which most likely drive the oscillatory motoneurons. On the assumption that one afferent fibre had only one encoding site, the more complex impulse patterns of the afferents could be splitted into impulse patterns of single encoders, all having roughly identical interspike interval distributions. The known impulse patterns of single encoders will be correlated with the impulse patterns of the intrafusal motoneurons in a following paper (18). Since one objective of this research is the understanding of the drive of the oscillatory firing extrafusal motoneurons, the afferent impulse patterns will be compared with the impulse trains of the oscillatory firing motoneurons (partly in this, but mainly in a following paper (17).

Secondary muscle spindle afferents branch to innervate the equatorial regions on one or both sides of the primary nuclear bag 1, nuclear bag 2, but mainly nuclear chain fibres (1, 4, 10). Since the branches become only unmyelinated 25-30 µm before their sensory terminals all main branches are myelinated (2, 6, 10). Ferric and ferrocyanide ions were used to stain specifically those sites where APs are initiated or propagated (11). Probable sites of APs origin were identified in the distal portions of Ia axons in neuromuscular spindles of cats and frogs (11). Terminal heminodes and some of the penultimate nodes are potential sites of spike generation (11). When muscle fibres in a spindle are stretched the final branches of spindle afferents are deformed and a generator potential (or algebraic sum of several receptor potentials (1)) is produced across the membranes of the terminals (9). This graded potential spreads electronically and excites one or more encoder sites to produce APs. The summed AP activity is then transmitted to the central nervous system via a single myelinated fibre. There has been quite a discussion about how single encoders contribute to the summed impulse activity of the parent axon, especially since in mammels it has not been possitive to precisely locate the encoders, because the distal afferent branches are bound together inside the capsule and are not accessible or separable with current physiological methods. When an impulse, propagating centrally in a first-order branch, reaches the branching node it will subsequently propagate orthodromocally into the parent axon and, provided that it is not refractory, antidromically into the first-order branch. During the subsequent refractory period this branch would then be less likely to transmit another impulse, thus suppressing its pacemaker. However, the antidromic impulse itself may fail to propagate into some of the pacemaker branches, or be eliminated by collisions with abortive orthodromic spikes within them. Since these effects would be cumulative, a profusely branching first-order system would recover from an antidromic impulse more rapidly than with fewer branches.

The single-fibre recording method from nerve roots or nerves is another approach to clarify the problem of the contribution of single encoder sites to the summed impulse traffic in the parent secondary muscle spindle afferents. By recording simultaneously from many fibres and comparing their impulse traffic, the simplest possible source of AP production is extracted. Probably the impulse patterns of such sources are the impulse patterns of single encoders. Since muscle spindles can be specialised according to their function in different

parts of the body (12) and encoder sites may influence each other, it is still possible that encoder fire with different impulse patterns.

Since impulse patterns of secondary spindle afferents of the accuracy presented herein are not available even from animals, in particular for direct comparison to physiologic α (extrafusal) and γ-motoneuron (intrafusal) impulse patterns (18), it will be shown that a recording from many fibres can be superior to single-fibre recordings.

Materials and methods

Measurements were done in a brain-dead human (HT6) and a paraplegic (intra-operative diagnosis during the implantation of a sacral root stimulator). For further details see previous publications (15, 16). On original recordings and schematic representations of impulse patterns afferent APs point upwards and efferent action potentials (APs) downwards.

Results

Identification of single muscle spindle afferents by the action potential wave form

From the muscle spindle afferent activity in a dorsal S4 root of HT6 the activities of 5 single fibres could be separated by their wave form and partly also by their firing pattern. The 5 fibres were the secondary muscle spindle afferents SP2(1), SP2(2), SP2(3) and SP2(5), and probably the tertiary muscle spindle afferent fibre SP2(4). Approximately 3 other muscle spindle afferents were contained in the root, but they were seldom active during the different stimulations. The activity of the 5 identified muscle spindles afferents, which contributed more than 95% to the muscle spindle afferent activity of the HT6 S4 dorsal root, and the identified activity of 2 secondary muscle spindle afferents from a S4 root of paraplegic 1, will be used to analyse the impulse patterns of the single encoding sites of secondary muscle spindle afferents.

Figure 1 shows that the 5 spindle afferent action potentials (APs) of HT6 could be safely distinguished from each other. Even though the APs of the SP2(2)-fibre could be safely distinguished from the APs of the other 4 fibres shown in figure 1, its APs looked rather similar to those of about 3 other fibres, not under consideration. The activity of the SP2(2)-fibre was mainly distinguished from them by the doublet firing shown in figure 1D. The APs of fibres SP2(1), SP2(2), SP2(3) and SP2(5) were conducting with velocities between 38 and 40 m/sec and are considered as secondary muscle spindle afferents based on the conduction velocity frequency distribution histogram constructed for HT6 (13). In SP2(5)-fibre the conduction from the distal electrode pair (trace b) to the proximal pair (trace a) was partly blocked (Fig. 1D), probably by some mechanical alterations. But occasionally also in this fibre AP was conducted across the two electrode pairs quite normally, so that the conduction velocity could be measured (38 m/sec).

The APs of the SP2(4)-fibre were conducted at 13.3 m/sec (Fig. 1E), much slowlier than in secondary spindle afferents. Fibres may fall outside of the normal velocity range. However since the activity of the SP2(4)-fibre was very constant (see later), this fibre propably was a tertiary muscle spindle afferent fibre. Since such a fibre has been detected here for the first time, its data were pooled with those of the secondary spindle afferents. Golgi tendon organ afferents are not at all or only little present in the lower sacral range, and they conduct with velocities between those of the primary and the secondary muscle spindle afferents (13).

Based on the different AP wave forms (Fig. 1) the APs of the 5 muscle spindle afferents could be identified in various situations, and the activity patterns of these 5 single fibres could therefore be simultaneously measured. The doublet firing of the SP2(2) and SP2(5)-fibres (sometimes also the SP2(3)-fibre) is a new observation and will be analysed later.

Original recordings of the two secondary muscle spindle afferents of paraplegic 1, identified by their wave form, have been presented in other papers (16, 17).

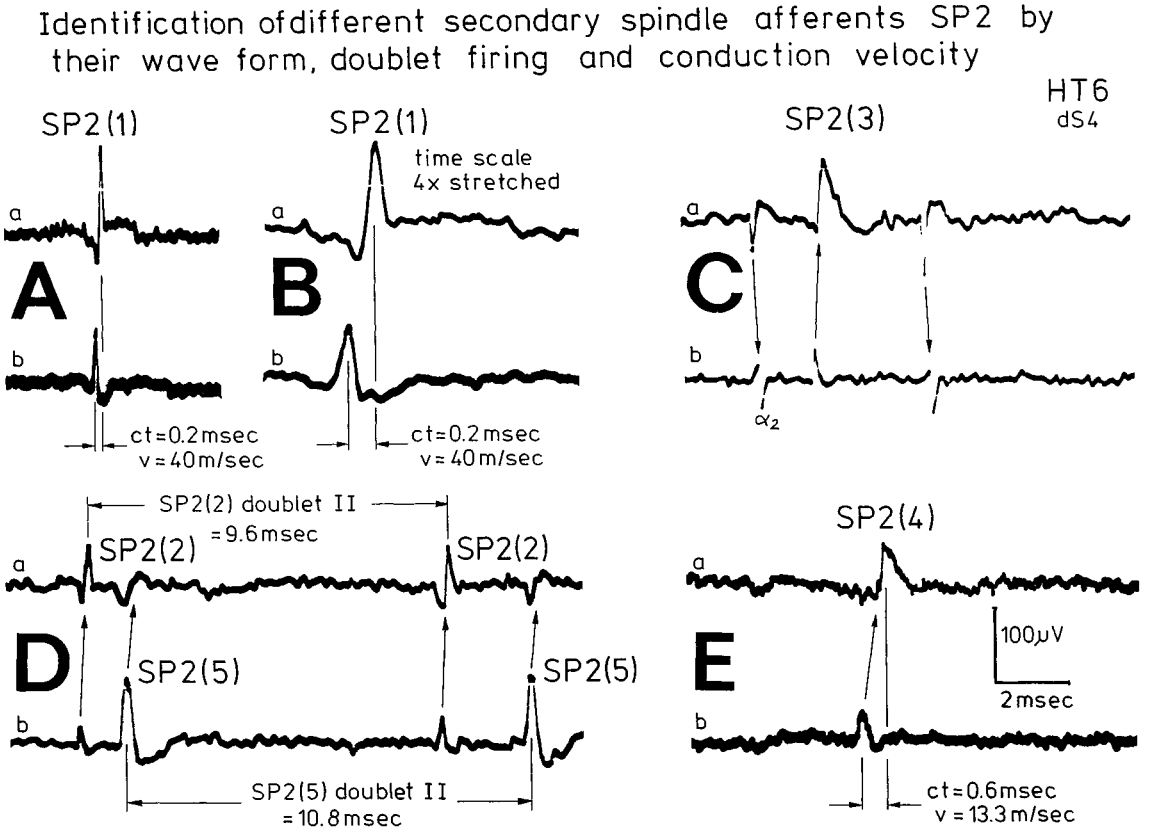

Fig. 1. — Extracellular action potentials (APs) of 5 secondary muscle spindle afferents SP2 (1 to 5) which are simultaneously identified by their wave forms. The SP2(4)-fibre (E) is likely a tertiary muscle spindle afferent, because of the low conduction velocity and the very little dynamic behaviour. The APs from the SP2(2)-fibre were sometimes identified by the doublet firing. II = interspike interval, ct = conduction time, v = conduction velocity, α_2 = α_2-motoneuron AP.

Single AP, doublet, burst and multi-ending regular firing of secondary muscle spindle afferents

Being able to identify the different secondary muscle spindle afferent APs, it is now tried to analyse the impulse patterns from single encoding sites. Figure 2A shows the distribution of interspike intervals (IIs) of the SP2(1)-fibre for anal and bladder catheter pulling, touch and pin-prick. It can be seen that the duration of the shortest II measured is 80 msec. Since figure 2A includes numerous IIs and responses to various kinds of stimulations, it is concluded that the duration of the shortest possible II is 80 msec for the constantly stretched anal sphincter (catheter ∅ = 12 mm). Since in addition, the distribution of the other spindle afferents (Fig. 2B, C, D) showed less specific properties and more intricate structure, the distribution in figure 2A is interpreted as originating from a single encoding site; this means that the SP2(1)-fibre possessed only one active encoding site. Based on this assumption or interpretation, it will be tried below and later on to understand the more complex impulse patterns of single secondary spindle afferent fibres with several encoding sites. The frequency distribution shown in figure 2A suggests that the single encoding site preferred certain IIs, in particular 80, 140 and 200 msec. The peak between 140 and 170 msec is splitted. It could be that this splitting is due to a hysteresis-effect following the increase and decrease of the contracture of the intrafusal muscle fibre due to different intrafusal activation. The oscillation periods of possible spinal oscillators, taken from the relationship between the oscilla-

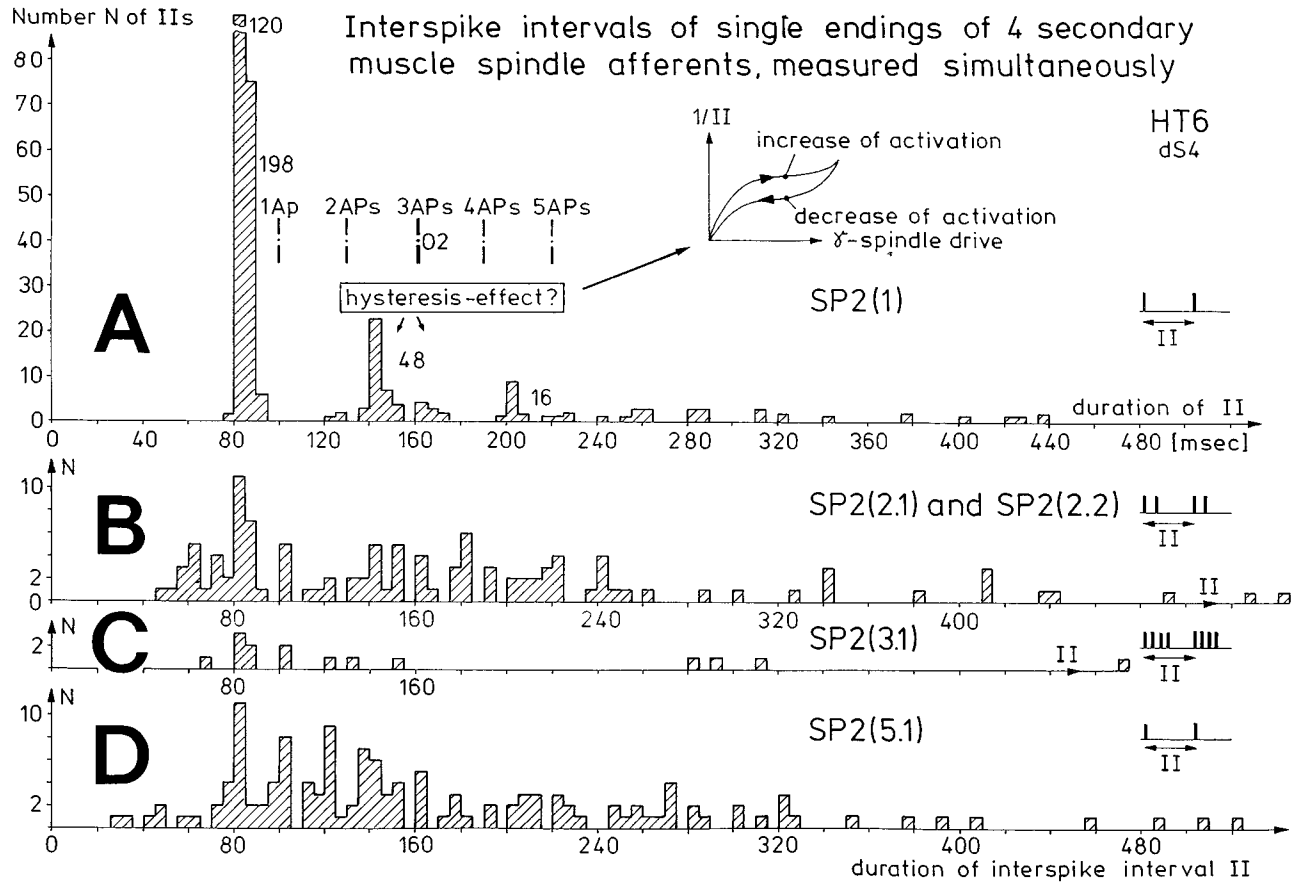

Fig. 2. — Interspike interval frequency distributions of single endings of 4 secondary muscle spindle afferents, measured simultaneously. In A, the theoretical oscillation periods are drawn for comparison. The oscillation period of the oscillatory firing α_2-motoneuron O2 of HT6 is also indicated. Note that the discretely distributed SP2(1)-IIs (A) are similar to the oscillation periods of the O2-oscillator for different impulse train length. The double peak at about 160 msec ay be due to a hysteresis-effect of the intrafusal muscle fibre (inset in A). The figure after the point in the parentheses (SP2(5.1)) indicates the single encoding site of a single secondary muscle spindle afferent.

tion period and the number of APs per impulse train (16) are indicated in figure 2A with 1 AP to 5 AP. The actually measured α_2-oscillator O2 is labeled 3 AP. It could be that due to the hysteresis-effect induced splitting of the II peak between 140 and 170 msec, the α_2-oscillator O2 is driven by IIs shorter than the oscillation period and is inhibited by IIs longer than the oscillation period. Probably, certain combinations of shorter and longer IIs could drive or not the oscillator. In a following paper comparing the afferent drive with the impulse activity of spinal oscillators, the hysteresis-effect will be picked up again (Fig. 4C of Ref. 17). However, the impulse patterns of the other spindle afferents (Fig. 2B, C, D) have also to be considered for the drive of the oscillator.

Assuming that an II of about 80 msec is the shortest II of a single encoding site, the impulse patterns of the single afferents SP2(2), SP2(3) and SP2(5) could be splitted into probable patterns of single encoding sites (Figs. 7, 8), and their II distributions were plotted in figure 2B, C, D. The very static fibre SP2(4) had such a low activity that no short IIs occurred.

The secondary muscle spindle afferents SP2(1) through SP2(5) showed four ways of firing: single-AP firing (SP2(1) and SP2(5)), doublet firing (SP2(2) and SP2(5)), burst firing (SP2(3)), and multi-ending regular firing

(SP2(2)). The muscle spindle afferents partly changed their way of firing according to the kind and strength of stimulation, and maybe according to the activity of the efferent parasympathetic innervation of the spindle (18).

It was assumed that the doublet firing originated in a coordinated activation of two encoding sites, and the II distribution for the doublet firing of the SP2(2)-fibre is shown in figure 2B. The distribution is similar to that shown in figure 2A. The II distribution of the SP2(2)-fibre is not as specific, and has values below 80 msec. Since in this case identification of the AP wave form was not as safe as for the SP2(1)-fibre, these shorter IIs could result from misjudged AP wave forms. This can be the reason only partly, since IIs shorter than 80 msec were also observed for the SP2(5)-fibre (Fig. 2D), and their APs could be identified safely. An explanation for IIs shorter than 80 msec is that in spindle afferents with more than one encoding site, there is some interaction between the encoding sites, so that the shortest II of a single encoding site is reduced.

The burst firing of the secondary spindle afferent fibre SP2(3) is analysed in a similar way. It is assumed that the burst firing originates from the activity of at least 4 encoding sites. The resulting distribution is shown in figure 2C. Figures 7 and 8 show the splitting of the burst firing, which resulted in the distribution shown in figure 2C.

The secondary spindle afferent fibre SP2(5) fired with single APs and with doublets. During the doublet firing, the activity was very low and the IIs were too long to provide information about shortest II. During the single-AP firing mode however, the activity was sometimes high and the II distribution of could be determined as presented in figure 2D.

Following the splitting into different encoding sites, the interspike interval (II) distribution were very similar, and the fibre with the expected single encoding site (SP2(1)) showed most specifity. The IIs of the secondary spindle afferents of paraplegic 1 showed similar distributions. Nevertheless, since the AP wave form identification was not as safe as in HT6, a splitting into single endings was not tried: just a few misjudged APs can change the distribution quite strongly.

The SP2(2)-fibre did not fire with doublets only. Following very strong bladder catheter pulling it fired with increasing high activity, with rather similarly spaced APs. Figure 5C shows the II distributions with the activity increasing from a through d (note the different scale in d). Assuming a shortest II of 80 msec for each encoding site, it is found that up to 6 encoding sites were activated with increasing activity. The firing was mostly regular with respect to the IIs. When a new encoding site became active, there occurred some irregularity in successive IIs, but quickly the IIs became regular again. This multi-ending regular firing is probably due to the parasympathetic innervation of the spindle, and will be analysed further in a subsequent paper (18). The recruitment of new encoding sites and the regularity of the firing pattern can be directly seen in figure 6A of Ref. 18.

Successive interspike intervals of secondary spindle afferents

Interspike interval (II) distributions provide information about the average occurrence of certain II durations, but no information about the decrease and increase of the IIs. Successive IIs of secondary muscle spindle afferents show directly, how quickly changes occurred. To turn out the dynamic properties of muscle spindle afferents is of interest for the understanding of the physiologic function of the spindles. But since in the anal sphincter and associated functions of the pelvic floor the extrafusal muscle fibres change their length only little, changes of the spindle afferent activity will be mainly due to contraction-associated changes of intrafusal muscle fibres. Changes of the muscle spindle afferent activity are therefore mainly due to changes of the activity of the efferent innervation of the spindles, which originates in the circuitry of the central nervous system. The aim of this research is to find out what is pathologic in the function of the spinal cord, in addition to

exploring normal functions, hopefully present in HTs. Comparisons between spindle functions of in subjects with paraplegy and in HT are expected disclose pathologic behaviour, if present.

Figure 3B shows 3 characteristic sets of successive IIs of the secondary spindle afferent fibre SP2(1) measured in HT. Set II shows the highest activation possible, namely firing with the always shortest II of 80 msec. In Sets I and III, IIs change from 140 and 260 msec to 80 msec and vice versa. The changes in the duration of successive IIs in paraplegic 1 (Fig. 3A) were much more pronounced. In set II there is a change from 240 msec to 25 msec. In general, the short IIs in the paraplegic subject were much shorter than in the SP2(1)-fibre measured in HT. The spindle afferents of paraplegic 1 seemed to be less stable and over-activated. From the previous section it is known that the SP2(1)-afferent fibre possessed only one encoding site with a rather simple impulsation pattern, whereas the SP2(1)-fibre of paraplegic 1 could have possessed several

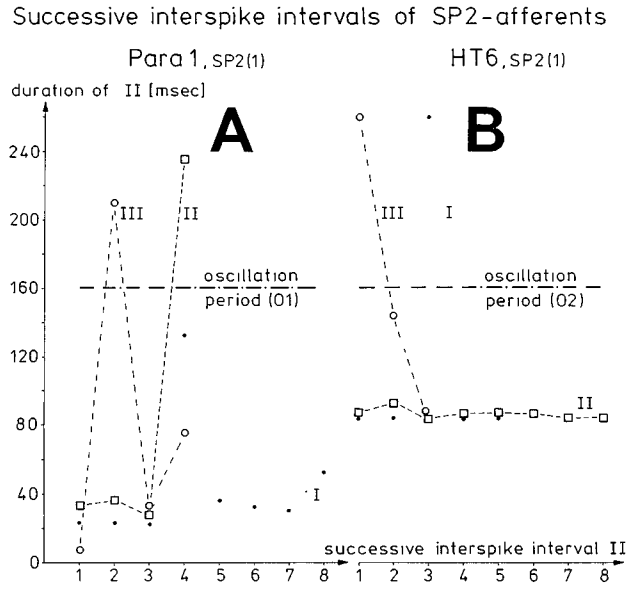

Fig. 3. — Successive interspike intervals (IIs) of single muscle spindle afferents of paraplegic 1 (SP2(1)) and the brain-dead human HT6 (SP2(1)). The figures I, II and III designate 3 series of successive IIs. The dotted and dashed lines link the points representing interspike interval values. The oscillation periods of the oscillatory firing α_2-motoneurons O1 and O2 are indicated.

encoding sites with higher and more structured activity of the parent fibre. The search for different encoding sites and the firing pattern will provide more information on whether the spindle afferent fibre of paraplegic 1 was really overactivated.

The oscillation periods of the continuously oscillatory firing α_2-motoneurons are indicated in figure 3A and B. The IIs of 130 msec and 140 msec duration could contribute to the drive of the oscillator.

Figure 4B (solid lines) shows successive IIs of the spindle afferent fibre SP2(3) which sometimes fired in a burst-like way. No regard was paid to different encoding sites. Sets I and II show similarity to the successive IIs of paraplegic 1 (Fig. 3A). Based on this comparison it is concluded that the secondary muscle spindle

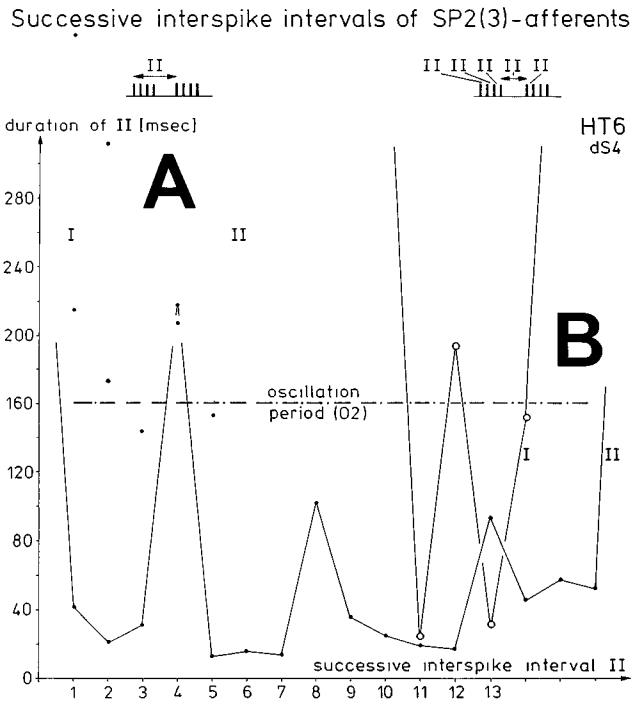

Fig. 4. — Successive interspike intervals of the secondary muscle spindle afferent fibre SP2(3). The way the interspike intervals of burst-like firing APs were measured are indicated. A. Two series (I, II) of single-ending IIs (dotted lines). B. Two series (I, II) of single afferents, but not of single endings (solid lines). Note that the single-afferent IIs are similar to those shown in figure 3A, and the single-ending IIs are similar to those shown in figure 3B. The oscillation period of the oscillatory firing α_2-motoneuron O2 is indicated.

afferent fibre SP2(1) of paraplegic 1 probably fired in a burst-like way. Pathologic function cannot be concluded from these sets of successive IIs. However, since in HT the fibre only showed burst-like firing following painful stimulations and the fibre of paraplegic 1 seemed to show always that kind of firing, it is probable that the afferent fibre of the paraplegic subject fired with a higher than normal activity. Considering now different encoding sites for the SP2(3)-fibre of HT6, successive IIs are obtained of the form shown in figure 4A (dotted lines).

Figure 5B shows successive IIs of the doublet firing of the secondary spindle afferent fibre SP2(2). The shape of the IIs makes up a saw-tooth pattern. Saw-tooth successive IIs are therefore an indication for doublet firing. Assuming that the doublet firing stems from the activity of 2 encoding sites, the successive IIs of the supposed single encoders are plotted in figure 5A. Mean IIs of the doublets are indicated at each set. The mean doublet IIs of 10.7, 11.4 and 11.6 msec seem rather similar. They will be further analysed below. As stated earlier, the SP2(2)-fibre was able to fire with much higher activity keeping all the time distances between the APs similar, as can be judged from the II distributions in figure 5C. Most likely, this coordinated activation of different encoding sites is due to the parasympathetic innervation of the intrafusal muscle fibres innervated by the SP2(2)-fibre. The parasympathetic innervation of muscle spindles will be analysed in connection with the somatic efferent innervation of muscle spindles in a following paper (18).

Doublet firing

The single-AP firing (SP2(1)-fibre) was the expected firing pattern. Burst-like firing was activated by pin-prick. Several encoding sites were probably activated for the response to

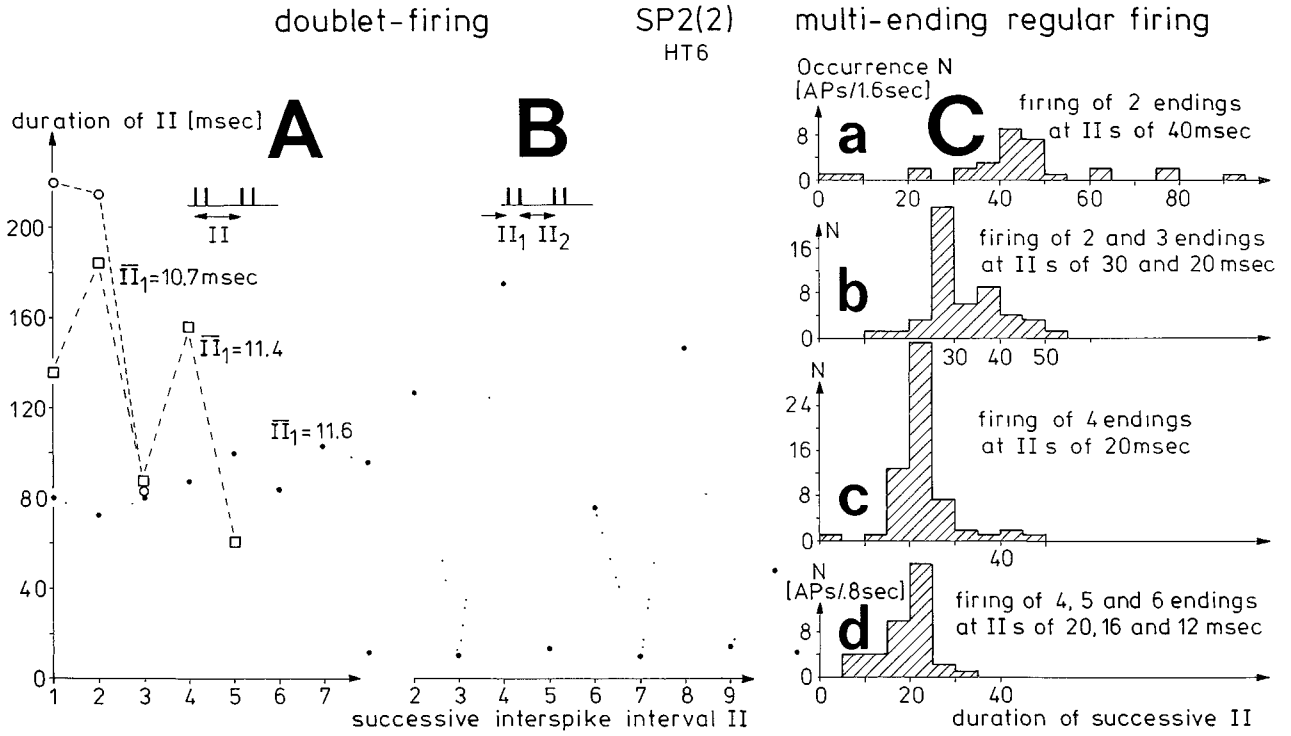

Fig. 5. — A., B. Successive interspike intervals (IIs) of the secondary muscle spindle afferent fibre SP2(2). The way of measuring is indicated. In A, 3 series of successive single-ending IIs are shown. $\overline{II}_1$ = mean doublet II. In B, saw-tooth like successive IIs are shown. C. Interspike interval frequency distribution histograms (a, b, c, d); increasing activity following bladder and anal catheter pullings. Peak suggests the number of activated endings, when the shortest single-ending II (80 msec) are devided by the value of the peak II (e.g. 80 msec: 40 msec = 2 endings).

pain, to serve the escape reaction. Multi-encoding site regular firing will be shown to be activated by parasympathetic fibres. A reason for the doublet firing is difficult to find, especially since the average activity level is rather low.

Figure 6 shows the distributions of the doublet IIs for the two secondary spindle afferents SP2(2) and SP2(5) during two series of measurements. At the end of the first series of measurements a very strong bladder catheter pulling most likely activated the parasympathetic system (Fig. 7 of Ref. 18). Some doublet firing was recorded in the first series of measurements from both afferents. More doublet firing was measured in the second series after the activation of the parasympathetic nervous system. One possibility is that the doublet firing is connected to the activity level of parasympathetic intrafusal motoneurons. A second possibility, as will be shown later, is that the activation pattern is caused by γ-motoneurons.

The doublet firing distribution of figure 6C shows three peaks at 10.2, 11.2 and 13 msec, represented by the solid line. Also, the three peaks are suggested on the distribution shown in figure 6A, which is based only on a few measurements. The doublet firing of these two afferents seems to have three sources. The 13 msec peak doublet is labeled γ_1 to indicate

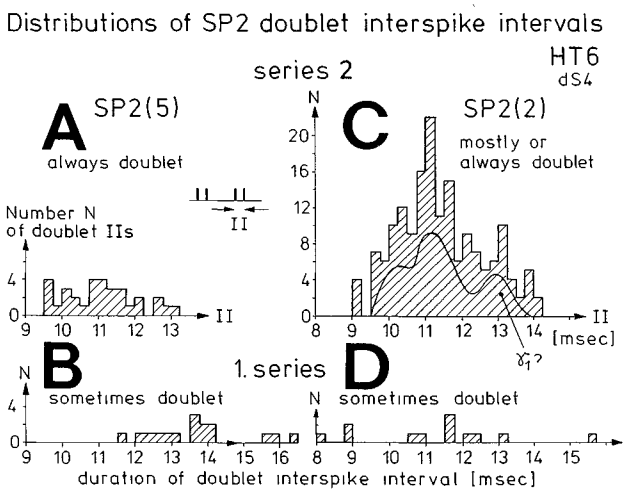

Fig. 6. — Doublet interspike interval frequency distributions of the secondary muscle spindle afferent fibre SP2(5) (A, B) and SP2(2) (C, D), HT6. The distributions in A and C may contain 3 peaks (C) one of which is labeled γ_1.

that a single γ_1-motoneuron (intrafusal) was partly firing with doublets, which also had a peak II duration of 13 msec.

Impulse patterns of secondary muscle spindle afferents in relation of the continuously oscillatory firing of α_2-motoneuron O2

In addition to interspike interval (II) distributions, durations of successive IIs and doublet II distributions, even more information can be obtained from impulse patterns by directly plotting the action potentials (APs) of the 5 spindle afferents vs. the impulse pattern of the oscillatory firing motoneuron.

In figure 7A, B, C the impulse patterns of the different fibres can be seen. Figure 7D shows the IIs between the first encoding site of the SP2(3)-fibre (SP2(3.1)) and the SP2(4)-fibre which is probably a tertiary muscle spindle afferent fibre. As the distribution in D shows, there is a correlation between these two impulse patterns. Thus, the afferent fibre SP2(4) belongs to the same spindle as the SP2(3)-fibre (in A, B, C indicated by the dashed box), or the SP2(4)-fibre belongs to a spindle, which is in tandem (12) with the spindle of the SP2(3)-fibre.

Figure 7A shows the activity of four spindle afferent fibres following light pin-prick 1. The activity of the SP2(1)-fibre is high, those of the other fibres are low. The open arrow marks a quick change of the oscillation period and a change of the impulse train. Such a quick change of the oscillatory firing was only observed once. Following strong pin-prick 2 (Fig. 7B) the SP2(1)-fibre increased its activity with a delay of about 510 msec, and the probably pain-sensivity SP2(3)-fibre fired transiently with bursts 250 msec after the pin-prick (Fig. 7B). Splitting up the SP2(3)-activity into the activities of the probable single encoding sites, two of them indicated by the dotted lines (labeled SP2(3.1) and SP2(3.2)), it can be seen that the burst-like firing can be explained by the recruitment of 4 to 5 encoding sites, in which the II decreased and increased in a similar way as for the SP2(1)-fibre. The SP2(4)-fibre was activated slightly later than the

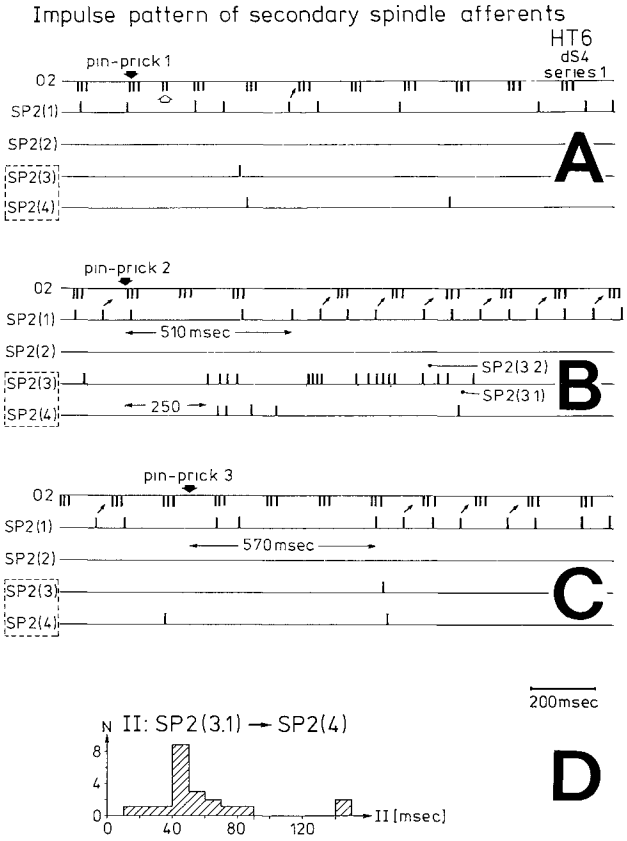

Fig. 7. — Impulse patterns of 4 simultaneously measured secondary spindle afferent fibres (A, B, C) following pin-prick. Each bar represents an AP; upward = afferent, downward = efferent. In B, the activity from 2 single endings (SP2(3.1) and SP2(3.2)) is marked by dotted lines. The small arrows indicate similar time intervals between muscle spindle afferent APs and motoneuron O2 APs. D. Interspike intervals between SP2(3.1) and SP2(4)-fibres.

SP2(3.1)-encoding site. With a shortest II of about 80 msec of a single encoding site, also the SP2(4)-fibre may have had more than one encoding sites. Following light pin-prick 3, only the activity of the SP2 (1)-fibre increased with a delay of 570 msec (Fig. 7C). The light and strong pin-prick were quantified in a previous publication (13) by measuring only pain afferent activity following the strong pin-prick.

Since it may be expected that during the constant stretch reflex (19) of the anal sphincter the spindle afferent activity drives the oscillatory firing α_2-motoneurons innervating the anal sphincter (14), similarities have to be looked for the IIs of the afferents and the oscillation periods, as was done previously (14), and for a rather constant phase relation between the afferent APs and the oscillation cycle, which is marked by the first AP of the impulse train. Assuming the existence of a phase relation for the drive of the oscillator, if the afferent AP leads 30 to 70 msec (mean $\pm$ s.d. $= 50 \pm 10$ msec, $n = 23$ of SP2(1) and SP2(2)) to the first AP of the impulse train, then the small arrows in figure 7 (and 8) from the SP2(1)-APs to the first AP of the motoneuron impulse train indicate phase relation. Driving phases of about 50 msec occurred 17 times between the SP2(1)-APs and the motoneuron impulse train, and 6 times between the SP2(2)-APs and the impulse train (Figs. 7, 8). As can be seen, most phase relations are present in figures 7, 8, following the painful pin-prick 2. It is reasonable that the anal sphincter enhanced its contraction following pin-prick into the gluteus maximus in addition to the constant stretch reflux induced by the anal catheter. In figure 7 it can be seen that the SP2(1)-fibre is almost the only one to contribute to the driving phase relation. Since in figure 7A there is only one afferent AP with the right phase, probably also other afferents, not contained in that S4 root, contributed to the drive of the oscillatory firing α_2-motoneuron.

In a second series of measurements, after the activation or increased activation of the parasympathetic fibres the impulse patterns of the 5 muscle spindle afferents were quite different, whereas the oscillator was still continuously oscillating. The activity of the SP2(1)-fibre is low (Fig. 8A, B) and nearly no phase relations occur for the motoneuron drive. Only following bladder catheter pulling 4 the SP2(1)-activity increased and more phase relations occurred. The activity of the SP2(2)-fibre was different from those of the first series. The SP2(2)-fibre fired now with doublets (Fig. 8A), and this doublet firing increased with the bladder catheter pulling (Fig. 8B, C). Also, phase relations (indicated by the small arrows) occurred now between the SP2(2)-APs and the impulse train of the motoneuron. There seemed to be also some coordination between the SP2(1)-fibre activity and that of the SP2(2)-

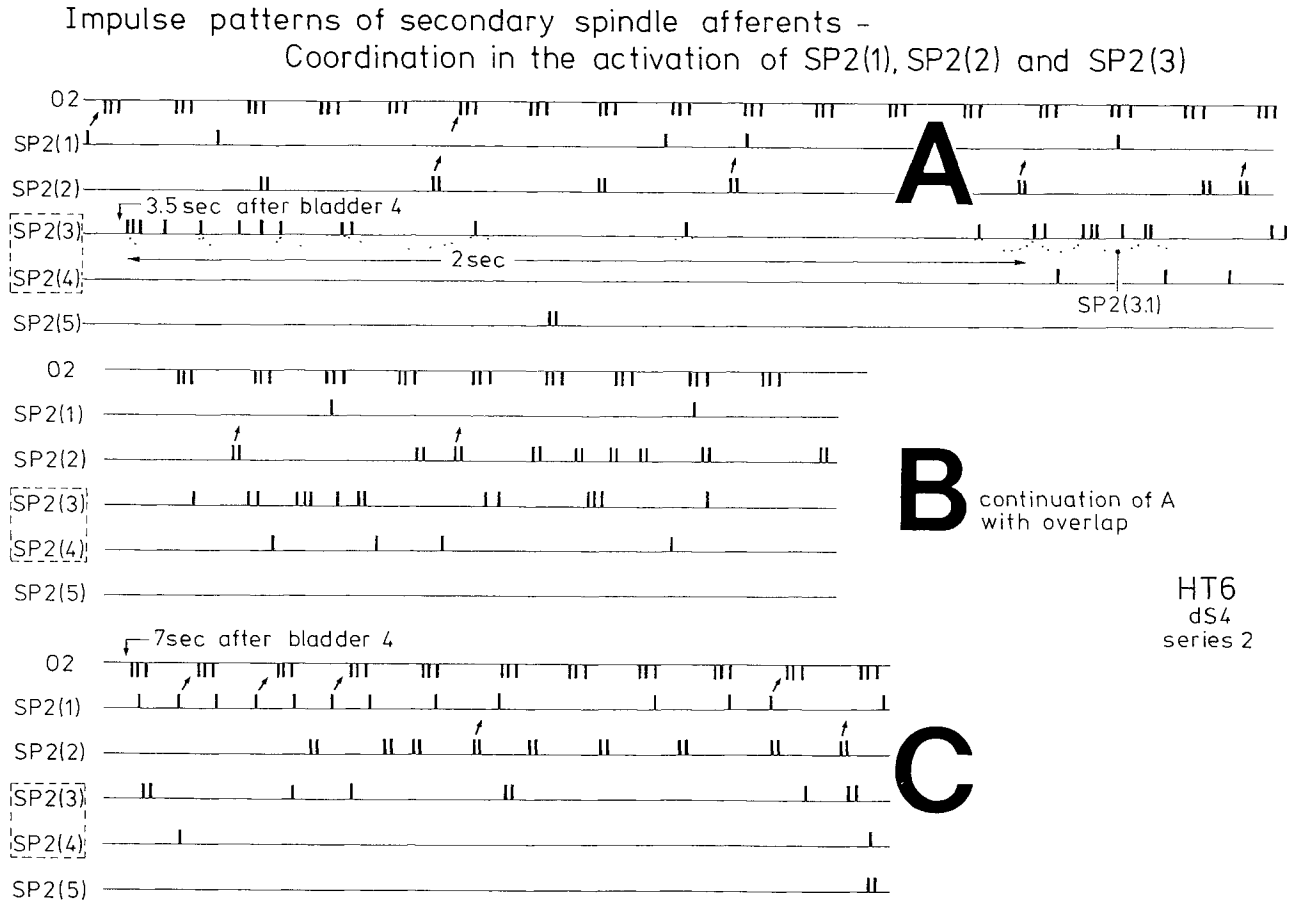

Fig. 8. — Impulse patterns of the 5 secondary muscle spindle afferent fibres SP2(1) through SP2(5) in relation to the impulse pattern of the oscillatory firing α_2-motoneuron O2 following bladder catheter pulling. Bars represent APs. Very short IIs (as in doublets) are not drawn to scale; only the first APs are in their exact position. The dashed rectangle around SP2(3) and SP2(4) indicates that both afferent probably innervate the same spindle; SP2(4) may by a tertiary fibre. The dotted curves, linking certain APs of the SP2(3) activity, most likely represent the activity from single endings. Note that doublet firing occurs in the spindle afferents SP2(2), SP2(5) and SP2(3); the doublet firing builds up in the SP2(3)-fibre from A (no doublet firing) to C (rather doublet firing). Note further the more static behaviour of the SP2(4)-fibre in comparison to the SP2(3)-fibre (delayed response, lower activity changes). The small arrows indicate similar time intervals from the afferent APs to the motoneuron APs (phase relation).

fibre, since only rarely their APs occurred at similar times. They shared the time for firing.

The activity of the SP2(3)-fibre strongly increased following strong bladder catheter pulling 4. Patients report the wish to micturate upon light till medium catheter pullings, and report pain upon strong bladder pullings. In figure 8A the activity of the most sensitive encoding site (SP2(3.1)) is indicated by the dotted line. The firing pattern was something in between repeated burst-like firing (Fig. 8A) and doublet firing (Fig. 8C). The SP2(3)-fibre preferred to fire when the SP2(1) and SP2(2)-fibres were silent. The activation of the SP2(3)-fibre was therefore included in the coordinated activation of the SP2(1) and SP2(2)-fibres. Possible phase relations are not indicated, since constant drive for the continuously oscillatory firing was looked for.

The activity of the SP2(4)-fibre also increased and it seemed to be also coordinated with those of the former spindle afferents. The SP2(4)-fibre was activated by painful pin-prick (Fig. 7B) and by painful bladder catheter pulling (Fig. 8A), similarly as the SP2(3)-fibre. The activity increase was delayed to that of the

SP2(3)-fibre, and was less pronounced. The activation of the SP2(4)-fibre was thus more static as compared to the SP2(3)-fibre. The SP2(5)-fibre fired seldom with doublets. Further comparisons between the activities of the secondary muscle spindle afferents and that of the oscillatory firing motoneuron will be presented in a following paper (17).

Discussion

Splitting of the summed activity into those of single encoding sites

It was shown in this paper that human secondary muscle spindle afferent fibres in the sacral range can fire with single action potentials (APs), with doublet APs, with AP bursts and with equidistant APs. Single fibres changed their mode of firing upon different stimulations. Afferent spindle activity increases occurred several seconds following stimulation. On the assumption that the secondary spindle afferent with the simplest impulse pattern (SP2(1)) possessed only one source of AP generation, the impulse patterns of the other spindle afferents were splitted into the patterns of single encoders. The justification of this splitting is given by the similarity of interspike interval (II) distributions (Fig. 2) of the constructed AP generation sources with those of the SP2(1)-fibre. Also, the decreasing and increasing of the encoding site IIs were similar as can be seen from successive IIs (Figs. 3B, 4A, 5A) and the direct impulse patterns (Figs. 7, 8). Further it will be shown in a following paper (18) that single γ-motoneuron II distributions are very similar to those of single encoders of secondary spindle afferents. In the multi-ending regular firing mode in the parent fibre the recruitment of a new encoder to further enhance activity was reflected by a transient irregularity (Fig. 6A of Ref. 18). In non-contracting muscles Vallbo measured IIs larger than 50 msec (21) and Burke et al. measured mean IIs of 90 msec (22).

Up to 6 encoder sites could be constructed from the impulse patterns of the SP2(2)-fibre (Fig. 5C) and up to 4 or 5 for the SP2(3)-fibre (Fig. 7); in a human muscle spindle 8 myelinated branches of primary afferents were found (10). In 4 cat hindlimb spindles 6 secondary afferent axons showed between 2 and 7 myelinated branches (1). With the finding that the APs are most likely generated in the myelinated branches at the heminodes or penultimate nodes (11), and assuming that the human secondary muscle spindle afferents in the sacral range are similar to those of the cat hindlimb, it follows that the number of encoder sites constructed from the impulse patterns is in accordance with the number (and range) of myelinated branches. It is therefore likely that the encoder sites constructed in these measurements were in the myelinated branches of the parent fibres.

Coordination of different encoding sites

Difficult to understand is, how the different encoder sites coordinate their firing. The burst-like firing of the SP2(3)-fibre (Fig. 7B) occurred because the 4 single encoders (SP2(3.1) through SP2(3.4)) fired in a somehow coordinated manner. An incoordinated firing of the 4 encoder sites would have looked differently. The doublets of the doublet firing of the SP2(2)-fibre (Fig. 8) showed a striking coordination. An easy explanation would be that the APs are generated in 2 myelinated branches. But how are the encoders coupled to give certain doublet II? Figure 6C shows doublets between 9 and 14 msec. The value of 9 to 14 msec cannot be explained easily by one encoder firing, antidromically invading the other side, and resetting the second encoder, since the value for the reset time is in the range of 1 to 2 msec (7). The doublet II distribution in figure 6C shows 3 peaks. The peak at 13 msec is labeled γ_1. This is to indicate that a simultaneously firing γ_1-motoneuron (intrafusal) in that nerve root fired with doublets, with a peak in the doublet II distribution also at about 13 msec (Fig. 4C of Ref. 18). Considering the lack of viscosity of nuclear chain fibres and that secondary afferents innervate bag 1, bag 2 and chain fibres,

but mainly chain fibres (1, 4), small fluctuations up to 60 Hz in polar length are readily transmitted to the equitorial region (1, 5); it could then be that doublet firing is due to the doublet firing of γ-motoneurons innervating the chain fibres. γ_1-doublets between 12 and 16 msec (18) correspond to frequencies between approx. 83 and 62 Hz. γ_{21}-doublets between 16 and 21 msec (18) correspond to frequencies between 62 and 48 Hz. Not before the impulse patterns of the efferent input (γ-motoneurons) to the muscle spindle fibres has been compared to the afferent output (spindle afferents) one should try to understand the impulse patterns of muscle spindle afferents, based on electronic generator potential spreading and concurrence of different encoder sites by antidromic invasion of one encoder site by the other and vice versa. First, it has to known what part of the structure of the afferent impulse patterns is due to the activity patterns of the efferent input. The remaining structure of the afferent impulse patterns, which is only due to the muscle spindle properties, should then be analysed with the competing encoder sites and electronic generator potential spread on the background of the detailed morphologic structure of the electrophysiologically measured spindles or similar units.

The doublet II distribution in figure 6C shows 3 peaks. If doublets were generated by 2 encoder sites, this could mean that 6 encoders contributed to the doublet firing of the parent fibre. The multi-ending regular firing suggested that the SP2(2)-fibre possessed at least 6 branches, possibly myelinated. The doublet II distribution peaks at 10.2, 11.2 and 13 msec may have occurred because of certain anatomical structures: e.g., the 2 branches producing the doublets may innervate different combinations of intrafusal muscle fibres, such as chain-bag 1, chain-bag 2 and bag 1-bag 2, or chain-chain, bag 1-bag 1 and bag 2-bag 2, or other combinations. Nevertheless, 3 sources of doublet AP generation were coordinated, since no IIs shorter than about 80 msec occurred.

If the doublet firing of the secondary muscle spindle afferents was generated by the doublet firing of the innervating γ-motoneurons, then 3 γ-motoneurons contributed, each one with its own doublet II distribution. It could also be that the γ-doublets generated different afferent doublets at different intrafusal muscle fibres. But since doublets occurred in γ-motoneuron and secondary spindle afferents, it could also be that not only the γ-motoneurons drove the spindle afferents, but also, at least in the pathologic case, the spindle afferents drove the γ-motoneurons and they in turn drove the spindle afferents. In the pathologic case a loop excitation may exist from the γ-motoneuron circuitry of the central nervous system to the muscle spindles. Oscillations between the γ-system and the muscle spindles have been suggested for some cases of pathologic tremor in humans (20).

It is reasonable to attribute some coordinated firing of different encoder sites of the secondary spindle afferents to the γ-systems. Figure 8 shows that the 4 parent afferents SP2(1) to SP2(4) fired mainly in a coordinated manner (3). Only seldom these fibres fired simultaneously. A coordianted firing of the different efferent endings is therefore likely. Progress in the understanding of the function of muscle spindles will come from further simultaneous measurements of the impulse patterns of the muscle spindle afferents and the intrafusal motoneurons, and from further morphologic details of human spindles in the body part of interest. The identification of impulse patterns of single γ-motoneurons measured simultaneously with the afferent impulse patterns was a key finding (18) for the understanding of the function of human muscle spindles; in addition, the parasympathetic activity was detected, since the γ-motoneuron impulse activity alone cannot explain the spindle afferent activity increase (Fig. 7, 8 of Ref. 18). As will be shown in the subsequent paper (18), the multi-ending regular firing of the SP2(2)-fibre (Fig. 5) is generated by the activity of parasympathetic muscle spindle innervation. Because of the still low level of parasympathetic activity, it has not been possible so far to safely pick up the pattern of the parasympathetic activity. A correlation between the activity pattern of the SP2(2)-fibre (Fig. 5C) and that of the parasympathetic axons to find a

reason for the multi-ending regular firing was therefore not possible. One possibility is that the recruited endings were unmyelinated and the heminode generated equidistant APs according to the addition of evoked generator potentials from the different activated endings.

The high complexity of the possible impulse patterns of parent muscle spindle afferents and the most likely high complexity of recruitment possibilities of the different encoding sites, including the mutual interactions, are in accordance with the high complexity of the afferent innervation of chain, bag 2 and bag 1 fibres and the probably complex firing patterns and innervations of intrafusal motoneurons.

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