Physiological Signs of the Activation of Bag$_2$ and Chain Intrafusal Muscle Fibers of Gastrocnemius Muscle Spindles in the Cat

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Taylor, A., P. H. Ellaway, and R. Durbaba. Physiological signs of the activation of bag$_2$ and chain intrafusal muscle fibers of gastrocnemius muscle spindles in the cat. J. Neurophysiol. 80: 130–142, 1998. A method is described for identifying the effect of single gamma static (γ$_s$) axons on bag$_2$, or chain intrafusal fibers using random (Poisson-distributed) stimuli. The cross-correlogram of the stimuli with the firing of spindle primary afferents took one of three forms. A large, simple, brief response was taken to indicate pure chain fiber activation and a small, prolonged response to indicate pure bag$_2$ activation. A compound response with brief and prolonged components was taken to be a sign of mixed innervation. The correlogram components could be well fitted with lognormal curves. They could also be transformed into curves of gain as a function of frequency, which were convenient for estimating the strength of the effects. In 68 effects of γ$_s$ axons on Ia afferents, 16 were pure chain, 17 pure bag$_2$, and 35 mixed. This distribution was significantly different (P < 0.05) from that expected from chance nonspecific innervation of chain and bag$_2$ fibers. Making use of the estimates of the strength of chain and bag$_2$ effects derived from the gain curves, the classification was modified by treating mixed responses that had one effect more than five times stronger than the other as belonging to the dominant type. The distribution was then as follows: chain 16, bag$_2$ 28, and mixed 24. This differed very significantly from the prediction of chance distribution (P < 0.001). This evidence for some degree of specific innervation of chain and bag$_2$ fibers is discussed in relation to previous work and with regard to the ways in which the two fiber types might be used in natural movements.

INTRODUCTION

The observations of Crowe and Matthews (1964) established that individual gamma motor axons supplying mammalian muscle spindles can be classified as one of two functional types, static or dynamic (γ$_s$, or γ$_d$). Although the characteristic actions of γ$_d$ axons are expressed through their specific actions on bag$_1$ intrafusal muscle fibers, the static action involves the activation of the other two types, namely bag$_2$ and chain (see Barker and Banks 1994; Boyd and Gladden 1985). The question arises as to whether the static fusimotor system actually has two functionally distinct parts corresponding to more or less separate innervation of bag$_2$ and chain fibers by distinct populations of γ$_s$ axons. The alternative is that the innervation of bag$_2$ and chain fibers is nonspecific and so ensures their combined activation in natural conditions. This is a very basic question that needs to be answered before the significance of the fusimotor system in movement control can be settled, but at present opinions are divided. Evidence suggesting independent innervation is the finding that changing fusimotor output by microinjection of picrotoxin into the substantia nigra could remove signs of tonic static output from primary afferents without affecting secondaries (Wand and Schwarz 1985). Also, Dickson and Gladden (1992) found that brain stem stimulation could differentially affect bag$_2$ and chain fibers in spindles exposed for direct observation. Detailed histological studies alone or combined with physiological recording have indicated in some cases a degree of selective innervation (Boyd 1986; Boyd et al. 1983), but in others a distribution of branches of individual static γ-axons to bag$_2$ and chain fibers that can be explained by chance innervation (Banks 1991, 1994a,b).

In designing experiments to answer this question, functional tests for the distribution of static axons to bag$_2$ and chain fibers are probably more relevant than morphological analysis, because the latter does not give any assurance as to the quantitative significance of collaterals of axons to particular fibers. Functional tests also have the advantage of providing information on large numbers of γ-axons in a variety of muscles, especially the larger ones, which are less suitable for histology. Functional tests for bag$_2$, or chain fiber innervation depend on the different mechanical characteristics of the two types. The fast twitch contraction and high tetanic fusion frequency of chain fibers cause them to drive the firing of Ia afferents with every stimulus or every second or third stimulus (Bessou and Pagès 1975; Boyd et al. 1977). This was exploited as the ramp frequency test in which signs of driving could be readily detected from comparison of instantaneous frequency records of the stimulus train and the Ia response at constant muscle length when the stimulation rate was raised linearly from 1 to 150 Hz in 2.5 s (Boyd 1986; Boyd and Ward 1982). Bag$_2$, fibers on the other hand do not cause driving because, having tonic characteristics, they contract only weakly and slowly for each individual stimulus and have a low tetanic fusion frequency (Boyd 1976). Since Boyd put forward his view that the ramp frequency test reliably distinguished between bag$_2$ and chain fiber activation and used its results to propose that there exists effectively separate innervation (Boyd 1986), there have been two important studies that examined the situation in more detail. First, Dickson et al. (1993) used the same test on a large group of static γ-axons supplying four different hindlimb muscles. Second, Celichowski et al. (1994) additionally sought to distinguish bag$_2$, and chain fiber effects on the basis of their different tetanic fusion frequencies. In both cases an important innovation was the use of cross-correla-
tion of the stimulus train with the afferent response to detect signs of chain activation with greater sensitivity and objectivity than is possible with the ramp frequency test alone. It has now been shown that the cross-correlation technique can be enhanced by replacing the regular stimulus trains by random, Poisson-distributed stimuli and greatly increasing the record lengths (Duruba et al. 1993; Taylor et al. 1995a, 1996). In this paper we present data obtained in this way from a population of $\gamma$-afferent axons supplying the medial gastrocnemius, a large muscle with well-defined function. We compare the various methods of distinguishing $b_1$- and chain fiber activation and present evidence in favor of a degree of specific innervation.

METhODS

General preparation

Cats (25, weight range 2.5–3.5 kg) were anesthetized with pentobarbital sodium (60 mg/kg ip) and were then maintained with supplements (12 mg/kg iv). A tracheal cannula was inserted to allow for artificial ventilation and to monitor end-tidal CO$_2$. The cephalic veins of each forelimb were cannulated for the delivery separately of anesthetic and other drugs. The right carotid artery was cannulated to monitor blood pressure and heart rate. Body temperature was monitored with a rectal thermometer and maintained close to 37°C with a feedback-controlled heating blanket.

The left hindlimb was denervated except for the nerve supplying the medial gastrocnemius (MG) muscle. This nerve was usually divided into two natural parts, and these were placed on separate pairs of silver wire hooks for recording and stimulating purposes. In some cases one of these nerve branches was cut so as to reduce the innervation. The muscle was separated from surrounding structures and its distal tendon attached to a servo-controlled electromagnetic puller (Lin Dynamosics) to provide controlled stretches. Electromyographic (EMG) wires were inserted into the MG muscle to check for $\alpha$-motoneuron activity during ventral root stimulation. After performing a lumbar laminectomy, a skin pool was formed around the exposed cord and filled with 3% agar gel in saline. When this was set, the center was scooped out to make a conventional paraffin-filled pool. The dura was then opened and dorsal and ventral roots of $L_7$ to $S_2$ cut close to the cord. A leg pool was also formed around the exposed gastrocnemius muscle and nerve, and both pools were kept close to 37°C by radiant heat. The temperature was monitored with thermistor probes adjacent to the sciatic nerve and under the gastrocnemius muscle.

Recording and identification of muscle spindle afferents

Muscle spindle afferents were isolated from dorsal root filaments and arranged for simultaneous recording from between 6 and 12 single units. They were characterized by their responses to muscle stretch, to twitch contraction, and by conduction velocity (CV), as measured by backward triggered averaging from the nerve close to the muscle. In addition, they were tested with succinylcholine (SCh; 200 $\mu$g/kg iv injection), to assess the influence on each afferent of the $b_2g$ $(b_1g)$ and $b_2c$ $(b_1c)$ intrafusal fibers. In this way it was possible to designate each afferent as one of the types $b_1c$, $b_2b+c$, $b_2c$, or $c$, given that all are known to contact chain (c) fibers. Full details of this procedure are given in Taylor et al. (1992a,b).

Isolation and testing of $\gamma$-axons

Ventral root filaments were stimulated and, if action potentials of gamma CV range could be recorded in the muscle nerve, were progressively subdivided until a single gamma unit was evident without alpha contamination. The gamma CV was calculated from the action-potential latency measured from averaged neurogram recordings. During subsequent stimulation, the muscle nerve record was continuously monitored to ensure that a single $\gamma$-axon continued to be active. Actions on the afferents were then sought by looking for effects on the ramp-and-hold stretch responses (5 mm in 1 s, repeated every 6 s) of stimulating the $\gamma$-axon at 100 Hz. Each effective $\gamma$-axon was then tested as follows.

1) Constant frequency stimulation at 30, 50, and 100 Hz and sometimes at 150 Hz for 15–30 s with the muscle length fixed. Each test was generally performed with the muscle length set just to take up the slack $(L_0)$ and with 4 mm and occasionally 8 mm additional stretch. Maximum physiological length was found to be $L_0 + 13.5$.

2) The above stimulation repeated in the presence of ramp-and-hold stretches starting from $L_0$ and from $L_0 + 4$ mm. The primary afferent responses to this were used to judge whether a $\gamma$-axon was static or dynamic, in the conventional ways (Crowe and Matthews 1964).

3) Ramp frequency stimulation (Boyd and Ward 1982). Axons were stimulated using trains of stimuli increasing in frequency linearly from 1 to 150 Hz in 2.5 s, with the ramp increase repeated every 6 s. Three or four ramp frequency stimulations were given at constant muscle lengths of $L_0$ and at +4 mm and in most cases at +8 mm.

4) Random frequency stimulation. The source for producing the random input triggers to the isolated stimulator was from a modified Geiger counter excited by a low-level radioactive source (natural uranyl acetate). The mean frequency was set to 50 or 25 Hz and a minimum of 5,000 stimuli delivered at each of the three muscle lengths.

Data recording, display, and analysis

Muscle spindle afferent spikes were discriminated and input together with stimulus trigger signals to a computer (66 MHz 486 PC) as times of occurrence using a CED 1401-plus interface with the Spike2 processing package (Cambridge Electronic Design, Cambridge, UK). They were displayed on the screen as instantaneous frequency in real time. In addition, the ramp displacement was also recorded sampled at 100 Hz. The data were analyzed offline using computer programs based on Spike2.

Construction of correlograms, spectra, and system gain plots

Cross-correlograms were constructed between the randomly delivered stimuli and the resultant afferent discharge (see Duruba and Taylor 1996). Usually, the correlograms had binwidths of 1 ms with pre- and poststimulus periods of 125 ms. They were normalized by dividing by the geometric mean of the numbers of events in the stimulus and response trains, so that the final display was the probability of firing of the afferent due to the presentation of the stimulus. The resultant probability distribution was fitted with a lognormal curve using “Kaleidagraph” (Abelbeck). The equation for this curve is

$$P(t) = A \cdot \exp \left\{ -\left(1/(2\sigma^2)\right) \cdot \ln^2 \left( [t - t_i]/(t_b - t_i) \right) \right\} + B$$

where $P(t)$ is the probability density, $A$ is the amplitude, $s$ is the standard deviation of the underlying normal distribution, $t_i$ is the time to the start of the curve, $t_b$ is the time of peak, and $B$ is the baseline value (Evans et al. 1993). In other cases, auto- and cross-correlograms were constructed using binwidths of 1/1,024 s and pre- and poststimulus periods of 512 ms. These were then Fourier transformed to produce the auto- or cross-spectra. The system gain, as a function of frequency, was computed by taking the cross-
spectrum and normalizing by dividing by the autospectrum of the stimulus signal. The gain could then be fitted by curves using Kaleidagraph. The curves used were derived from the expression for gain as a function of frequency in a first-order lag system, namely

$$G(f) = \frac{k}{f + (1 - f/f_c)^2}$$

where $k$ is zero frequency gain and $f_c$ is the corner frequency (−3 dB point). The interpretation of gain plots as computed in this way from neural spike data raises various problems that will be discussed later. To make an objective estimate of the start time ($t_s$) of the correlogram deflections, cumulative sums of differences from the baseline (CUSUMs) were calculated as described by Ellaway (1978), using 0.2-ms bins. CUSUMs were also computed with 1.0-ms bins to detect weak effects.

RESULTS

A total of 246 spindle afferents were recorded, of which 169 had CV ≥70 m/s and so are provisionally taken as primary afferents. Those primaries showing effects from the γ-axons studied numbered 58 and on testing with SCh, 42 of these were b1b2c and 14 were b2c type (2 unidentified). A total of 266 γ-axons were isolated, of which 95 showed effects on ≥1 afferent, primary or secondary. Of these γ-axons, 83 were static and 12 dynamic. The low ratio of dynamic to static (1:7) is not thought to be a result of some biasing in the sampling procedure, because the γ-axons were sought on the basis of CV and then tested individually for their effects on spindle afferents. Eighty-two of 95 γ-axons were tested completely, and of these 71 were static and 11 dynamic in their effects. This paper is concerned with the 54 static γ-axons that affected primary afferents (68 pairs), the identification of their contacts with bag2 or chain fibers, and the analysis of their distribution. The first concern is to consider the reliability of the driving phenomenon for indicating chain activity. We then proceed to examine the validity of the random stimulation method and how its results can be expressed quantitatively. Finally, data on the distribution of axons to bag2 and chain fibers are examined statistically for evidence of specificity of innervation.

Strength of driving caused by different static fusimotor axons

Primary afferent driving by ramp frequency stimulation of a static fusimotor axon can be seen in its most marked form as a reliable 1:1 relationship with very little variability up to 150 Hz (Fig. 1A1). It is worth noting that, even in the case of this exceptionally strong driving effect, driving does not become established until the stimulation frequency exceeds the ongoing afferent discharge frequency. Before that, the gamma stimuli only produce an irregular additional discharge. In other cases, the time locking is not so clear at any time and does not extend to such high frequencies (Fig. 1A2). The example in Fig. 1A3 shows reliable driving apparently failing above ~20 Hz, but there is evidence that the effect actually continues in some degree to ~100 Hz, in that the lowest frequency of the afferent during the ramp stimulation follows the ramp frequency. The presence of driving in the record in Fig. 1A4 could well be overlooked, but superimposing lines representing subharmonics of the stimulus frequencies indicates that the afferent firing actually fluctuates between 1:2, 1:3, and 1:4 in relation to the stimuli. Figure 1A3 shows a unit with no trace of driving. The afferent firing frequency rises along a smooth curve with no simple relation to the stimulus ramp. This gamma unit’s action was confirmed to be purely static during ramp-and-hold stretching.

There can be no doubt regarding the involvement of chain fibers when driving is clearly present. However, there is a possibility that weak chain effects might be missed, especially if they are accompanied by bag2 activation. As pointed out by Dickson et al. (1993), the recognition of the presence of driving is facilitated by computing correlograms between the ramp stimulus train and the afferent spikes. Figure 1B shows correlograms derived in this way from a minimum of three ramps, excluding the plateau periods of constant stimulation at 150 Hz. It is clear that the presence of any visibly detectable driving in the ramps is accompanied by a peak in the correlogram. The conspicuous side lobes in the correlograms of Fig. 1, B1 and B4, are related to regular elements of the stimulus train, but also depend on the presence of regular behavior on the part of the afferent, because the irregularly firing units 2 and 3 do not show them. The use of ramp frequency stimulus trains precludes any formal analysis of these correlograms. A development of the correlogram method using regular stimulation at 100 Hz was described by Celichowski et al. (1994). The results of applying this are shown in Fig. 1C with the modification that here, to be consistent with B, the period from −25 to +50 ms with respect to the stimuli is shown rather than just +10 ms. Now, of course, the regularity of the stimuli ensures that the peaks in the correlogram repeat endlessly at 10-ms intervals. In this respect, there is no difference to be seen between gamma effects 1–4, although with the ramp frequency stimuli the correlograms in two of these cases (1 and 4) showed side lobes, but 2 and 3 did not. It appears that some additional information is provided by the ramp stimuli, which is not elicited by the continuous 100-Hz stimulation.

Random stimulation

Although it may be possible to reach a reliable conclusion in each case as to whether there is any chain fiber activation on the basis of the above tests, we still lack a test that can objectively assess the presence and strength of chain effects and in addition does the same for bag2 effects. This appears to be possible using randomly spaced stimuli with Poisson-distributed intervals. Because the autocorrelogram of such a sequence is an impulse function at the origin and a constant value elsewhere, the cross-correlogram will not be expected to show side lobes (see Taylor et al. 1995a). In Fig. 2 the results are shown of applying this method to the same five units as illustrated in Fig. 1, using a mean stimulus frequency of 50 Hz. In Fig. 2A the instantaneous frequency of the afferents is shown for a 5-s control period and the first 15 s of random stimulation. It is notable that the afferent in row 1, which has a resting frequency of 55 Hz, shows peaks of frequency up to 500 Hz during stimulation but is also forced to drop well below its control level. This is no doubt associated with the very strong 1:1 driving in this case (Fig. 1A1).
INNERVATION OF BAG₂ AND CHAIN INTRAFUSAL FIBERS

A similar, but less marked effect is evident in unit 4 and in this case also driving is well time locked, although at subharmonics. Gamma axons in rows 2 and 3 are alike in causing peaks of frequency up to ~160 Hz with the impulses concentrated in a band well above the control, whereas the impulses in rows 1 and 4 are concentrated about the control frequency. The γ-axon in row 5 is quite different in that it causes only a small increase in mean frequency with relatively little irregularity. As in the other tests, all the γ-axons except for number 5 cause a distinct peak in the cross-correlogram. This peak is largest in the case of number 1, which causes the clearest 1:1 driving. There is no sign of sidelobes, and the use of 5,000 stimuli has evidently ensured a very smooth baseline and well-defined response. In addition to the large peak, the correlograms of rows 2 and 3 show an appearance of a second small, slow elevation. As a basis for further discussion, it is proposed that the random correlograms can be interpreted as follows. The relatively large fast peak is caused by contraction of chain fibers, and its absence from the response to stimulation of a static γ-axon indicates a pure bag₂ effect (the S type of Celichowski et al. 1994). Behavior as in rows 1 and 4 indicates pure chain effects (F type), strong and weak, respectively. The effects caused by γ-axons in rows 2 and 3 can be interpreted as due to combined contraction of bag₂ and chain fibers (M type). The second, smaller peak in the correlograms probably reflects activation of the bag₂ fibers. A small peak of this form might also be expected to appear alone in cases of pure bag₂ effects. Form of the random correlograms

The simplest form of correlogram, namely that illustrated in Fig. 2, A1 and B1, was observed in 11 of 68 γ, actions on primary afferents. In this instance, the waveform arises abruptly from a flat baseline with a lag relative to the stimuli of 12 ms, reaches a peak value of 0.38 (units of probability per ms) in ~2 ms, and declines more slowly along a smooth curve back to the baseline in ~6 ms. To provide objective measures of the strength and timing of the correlograms, it is useful to fit them with an analytically defined curve. An arbitrary function often used for asymmetrical time-varying processes is the α-function, but as shown in Fig. 3, B and D, this cannot provide a good fit. An alternative is the lognormal probability density function (Evans et al. 1993), which fits extremely well, given defined values for the baseline and the arrival time. In Fig. 3, A and C, two examples have been fitted after computing the mean baselines from the records 100 ms before time 0 and the starting times (t₀) from extrapolations of the CUSUMs to 0. The goodness-of-fit seen in these two examples is typical of the 11 correlograms of this type (the ρ² values ranging from 0.90 to 0.98) and provides objective estimates of the rise time (tᵣ = t₀ - tₑ) and the amplitude (A) and width of the curve at 0.5 amplitude (τᵣ). The starting time t₀ is the sum of the conduction times in the γ and the Ia axons between the recording point on the muscle nerve and the spinal roots, together with some component (tₑ) due to the processes within the spindle and the intramuscular conduction times. Mean val-
FIG. 2. Analysis of static gamma effects by random stimulation. The pairings of static $\gamma$-axons and Ia afferents 1–5 are the same as in Fig. 1. Their behavior is shown in response to Poisson-distributed stimuli with mean frequency of 50 Hz. $A$ shows the instantaneous frequency of primary afferent firing with 5 s of control followed by 15 s of stimulation. $B$ shows the cross-correlograms between the stimulus and response pulses using 5,000 stimuli and time bins of 1 ms. Ordinates are in units of probability of firing in each bin derived by dividing the counts per bin by the geometric mean of the number of stimuli and the number of response spikes. Note that the ordinate scale for units 2–5 is expanded 2.86 times relative to that for unit 1.

Values for these measures are shown in Table 1. There were five additional cases of weak driving that have been excluded from this table because their correlograms showed late components dipping below the baseline, as in Fig. 2B4 (see also Fig. 6G).

In marked contrast to the static gamma effects illustrated in Fig. 3, there are some with no sign of driving and much smaller peaks in the random correlogram. Two of these are illustrated in Fig. 4, one of them ($A$) being taken from row 5 in Figs. 1 and 2. Although the correlograms have been displayed here at higher sensitivity, it is doubtful whether any significant deflection could have been detected for $A$ but for the use of the CUSUM (Fig. 4B). The detectable, but still very small peak for another unit in $C$ again is made much clearer by the CUSUM in $D$. It proved impossible to fit a curve to the data in Fig. 4A, but the filtering effect of the integration process in the CUSUM improves the signal-to-noise ratio so much that a lognormal curve can now be fitted very effectively (continuous line in $B$). This noise-free fitted curve can then be differentiated and the control mean level added to give the continuous line as a fit in $A$. The same procedure has been followed in $C$ and $D$. There were 12 static gamma effects of this kind, in which a small, slow elevation could be detected in the correlogram, or with the aid of the CUSUM, and their parameters are summarized as the “slow” group in Table 1. The much smaller mean amplitude and longer $t_{0.5}$ for this group compared with the “fast” group (Table 1), and the lack of any other sign of driving strongly supports the view that they represent cases of pure bag effects. In five other cases, although the CUSUMs undoubtedly showed significant deflections, the signal-to-noise ratio was too poor to allow confident curve fitting.

The third type of random correlogram observed is exemplified by rows 2 and 3 in Fig. 2. In these cases the initial sharp peak is followed by a smaller, slower elevation, which suggests the presence of a second, slower component. This interpretation is supported by the curve fitting shown in Fig. 5 in which $C$ and $D$ are derived from the unit of row 3 in Fig. 2, whereas $A$ and $B$ come from another unit, not previously illustrated. In $A$ and $C$ of Fig. 5, the continuous lines are the curve fits obtained assuming the sum of two lognormal curves with the same values of starting times ($t_{s1} = t_{s2}$ as shown in the inset). The quality of the fits obtained is clearly very satisfactory, and the component curves are plotted superimposed on the data points in $B$ and $D$ together with the derived parameters of the curves shown in the insets. The
mean parameters of the first (fast) and second (slow) peaks are shown as the “mixed” group in Table 1.

The subjective judgment as to the presence or absence of a second component in the correlograms could usually be made confidently and supported by the curve fitting. However, in a few cases in which it appeared that a small second component might be present, the fitting algorithm was unable to estimate reasonable parameters for it. These cases were resolved by the use of plots of gain as a function of frequency, which are described below.

Comparison of the values for the fast and slow components estimated from the pure effects with those from the mixed effects show some clear differences. The mean amplitudes of both peaks are significantly greater in the pure than in the mixed effects. Neither the rise time nor the half-width of the fast components differ in the two situations, but both values are much longer for the slow component in the mixed cases than when this occurs alone. This suggests that a prior impulse initiated by chain contraction alters the responsiveness to bag₂ contraction. The value of \( t_m \) is 2.01 ms longer for the slow component than the fast one estimated separately. Presumably this reflects the slowness of the onset of contraction of bag₂ as compared with chain fibers.

The mean CV for gamma axons with pure bag₂ effects was found to be 7.03 m/s greater than for those with pure chain effects, a difference significant with \( P < 0.001 \) (see Table 1). Using 171 mm as the mean value for gamma conduction distance from ventral root to the point of entry into the muscle, we can calculate that the mean gamma conduction time is 1.41 ms less for bag₂ innervation than for chain. This largely compensates for the slower intramuscular events for bag₂ effects so that the mean value for \( t_m \) is only 0.52 ms greater for bag₂ effects than for chain effects. This is a reasonable justification for setting \( t_m = t_c \) when computing the best fit curves for the mixed examples, as explained above. \( \gamma \)-Axons with mixed effects have a mean CV intermediate between the other two groups.

The obvious interpretation of the above observations is that a \( \gamma \)-axon terminating on chain fibers alone in a given spindle will give rise to a correlogram with a single large, brief component, reflecting the fast-twitch characteristics of the chain fibers. A \( \gamma \)-axon acting solely on a bag₂ fiber will produce a much smaller and slower peak because the tonic nature of the fiber means that the individual stimuli will cause only very small and slow mechanical effects. Finally, an axon supplying both chain and bag₂ fibers will result in a correlogram with two components. Although the evidence for the above interpretation is circumstantial, the only real doubt that arises concerns the second peak of the complex correlograms. It has been suggested that a second peak may arise during regular stimulation as a result of two impulses being generated by the rising phase of the intrafusal chain fiber twitch (Emonet-Dénand et al. 1997). The other possible complicating factor is the presence of some degree of regularity in the primary afferent firing. This might be expected to produce an increased probability of firing following a chain fiber-related peak in the cross-correlogram at a lag time equal to the natural firing period. One way of looking for signs of these two effects is to examine the autocorrelograms of the afferent firing records during the period of stimulation.

Figure 6 shows data for three \( \gamma \) actions giving simple, relatively large fast peaks in the random cross-correlograms (A, D, and G). They were all thought to represent pure chain actions from this and from their ramp frequency responses. In the top row the clearest example of 1:1 driving shows an autocorrelogram (B) that rises abruptly to an almost constant value, with the exception of a brief initial peak. The autocorrelogram of the Poisson stimulus itself (not shown) consists of a unit pulse at time 0 followed by a constant value. In B, E, and H the initial pulse occupying the first bin has been

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**TABLE 1.** Summary statistics of mean values of estimates derived from curve fitting of the correlograms

|                | Fast           | Slow           | Mixed          |               |               |
|----------------|----------------|----------------|----------------|----------------|
| Amplitude (A)  | 0.150 ± 0.094  | 0.022 ± 0.014  | 0.093 ± 0.045  | 0.013 ± 0.006  |
| Rise time (\( t_1 \)) | 2.54 ± 0.70   | 3.66 ± 2.76    | 2.60 ± 0.91    | 14.76 ± 7.25   |
| Half-width (\( t_{0.5} \)) | 3.67 ± 1.22  | 15.10 ± 16.68  | 4.29 ± 1.33    | 35.71 ± 13.62  |
| Intramuscular time (\( t_m \)) | 4.45 ± 1.40  | 6.46 ± 2.27    | 4.67 ± 0.86    |               |
| Gamma CV, m/s  | 26.72 ± 4.01  | 33.75 ± 4.44   | 31.43 ± 5.04   |               |

Values are means ± SD. Amplitude A is in units of probability per ms. Number of values for Fast was 11, for Slow was 12, and for Mixed was 32. CV, conduction velocity.
FIG. 4. Two examples of correlograms from units showing no signs of driving. A and B are derived from the unit of row 5 in Fig. 1, whereas C and D come from another unit (not previously illustrated). A and C: correlograms with conditions as in Fig. 2. B and D: cumulative sums (CUSUMS) of the correlograms computed using the mean level in the 100 ms before the stimuli as the zero. The CUSUMS have been fitted with lognormal curves (continuous lines), which have then been differentiated to give the smooth curves superimposed on the data dots in A and C.

FIG. 5. Two examples of correlograms requiring two lognormal curves for adequate fitting. C and D are derived from the γ-1α pair of row 3 in Fig. 2, whereas A and B come from another pair (not previously illustrated). Dots show the original cross-correlograms with conditions as in Fig. 2, and the continuous lines are the computed curve fits using the sum of two lognormal curves. Total curve fits are shown in A and C, and the two component curves in B and D.

FIG. 6. Comparison of cross- and autocorrelograms and gain plots for three pure chain effects. Each row shows results from one static gamma effect. Top row: 1:1 driving. Middle row: 1:2 driving with no rhythmicity. Bottom row: 1:2 driving with rhythmicity. A, D, and G: cross-correlogram with Poisson input at mean frequency of 50 Hz, 5,000 stimuli, and 1-ms bins. B, E, and H: autocorrelograms of the afferent firing under the same conditions. C, F, and I: gain as a function of frequency computed as described in METHODS. Data points have been fitted with curves predicted for single 1st-order lag dynamics. Curve fit in I contains an additional Gaussian pulse to accommodate the rhythmic firing.

Clear evidence of regular afferent firing and the smooth dip after the peak in the cross-correlogram (G) is no doubt a reflection of this. E shows no sign of rhythmicity and, correspondingly, no such dip in the cross-correlogram (D). Thus there is no evidence to suggest that double firing or resetting of rhythmic firing are responsible for the second, small components in the cross-correlograms. The assumption is, therefore, that such peaks may be attributed to bag action.

Gain functions

In a linear system energized with white noise, the cross-correlogram of input with output yields the impulse function. In this case an alternative way of presenting the information contained in the correlograms is in the form of plots of gain as a function of frequency (see METHODS). This method of analysis can be helpful in understanding the system dynamics and in predicting behavior with other inputs. Figure 6, C, F, and I, shows three examples in which gain plots are shown on log/log scales. The general form of the curves suggests a first-order filter characteristic, and therefore continuous lines representing the best fitting first-order lag dynamics have been fitted. In C, the case of 1:1 driving, the fit was very good and gave a corner frequency \( f_c \) of 144 Hz and a zero frequency gain \( k \) of 0.77. The middle and bottom rows of Fig. 6 are examples of 1:2 driving, one with no sign of rhythmicity \( (D−F) \) and the other with a tendency to fire with a period of \( ~30 \) ms \( (G−I) \). The autocorrelogram of the afferent firing shown in \( E \) takes the form expected when a Poisson train is divided by 2 and modified by a dead time of \( \sim 2 \) ms. Here also the gain plot in \( F \) is well fitted by a first-order lag, now with \( f_c \) of 56.6 Hz and \( k \) of 0.71. At first sight, it is anomalous that the value of \( k \) in (1:2
driving) should be nearly as high as that in C (1:1 driving). In fact with 1:1 driving a value of \( k = 1 \) might have been expected, but this cannot be achieved because the dead time causes the loss of some of the random pulses. With 1:2 driving, \( k \) should theoretically be reduced from unity to \( 1/1/2 \) or 0.707, which is very close to that observed. In this case the dead time does not have so much effect because, with the Poisson train effectively divided by 2, few pulses are lost through this cause. In the bottom row of Fig. 6, there is 1:2 driving and marked rhythmicity as shown by the oscillations in the autocorrelogram. The cross-correlogram in \( G \) has a smaller peak than in \( D \), followed by a trough and small waves with 30-ms period. The gain curve is also generally well fitted by a first-order lag with \( f_c = 59.6 \) Hz and \( k = 0.35 \). The rhythmicity appears in the gain curve as a peak at 33.1 Hz, which has been fitted arbitrarily by the addition of a Gaussian pulse to the lag curve. The reduced low-frequency gain in \( I \) relative to that in \( F \) is probably related to the underlying regular discharge that interrupts the longer intervals occurring in the Poisson train.

Figure 7 presents further data from two static gamma effects that appeared to be mediated purely through \( b_2 \) fibers (see Fig. 4). Although the effects in the random cross-correlograms in \( A \) and \( D \) are weak, the gain plots in \( C \) and \( F \) are perfectly clear and well fitted up to \( \approx 30 \) Hz by first-order lag curves with \( f_c = 3.44 \) and 8.85 Hz and \( k = 0.16 \) and 0.30, respectively. Both gain plots are distorted at frequencies above \( \approx 30 \) Hz because of the regular firing, as indicated by the autocorrelograms in \( B \) and \( E \).

It appears from the above that the gain plots derived from the random cross-correlograms reflect in a simple way the different properties of the \( b_2 \) and chain fibers. Static gamma effects having characteristics implying chain action alone (ramp driving and large, brief cross-correlograms) give gain curves starting from high values at low frequencies and falling as predicted for a first-order lag with \( f_c \) around 50–100 Hz. Effects most readily explained by \( b_2 \) action are also fitted by first-order curves, but with \( f_c \) in the range 2–8 Hz. It is also evident that when an afferent has any natural tendency to fire rhythmically in the presence of the random gamma stimulation, this shows itself as a peak on the gain curve centered at the preferred frequency (see Matthews 1997). These observations help in the interpretation of those static gamma effects that give random cross-correlograms with two peaks. Figure 8 shows two such effects displayed in the same way as in Figs. 6 and 7. The cross-correlograms \( A \) and \( D \) show large, fast peaks that are undoubtedly due to chain action, but in addition second small, slower components. These seem likely to be due to \( b_2 \) action, but they might also be explained by the underlying rhythmicity reset by the gamma stimuli. Examination of the gain curves in \( C \) and \( F \) show values of gain at low frequencies even higher than for pure chain effects in Fig. 6 and apparently falling in two stages. These curves are not at all well fitted by single first-order lag responses, but rather require the sum of two such curves: in \( C \) one with \( f_c = 4.24 \) Hz and \( k = 0.68 \), the other with \( f_c = 29.9 \) Hz and \( k = 0.53 \) and in \( F \) one with \( f_c = 4.92 \) Hz and \( k = 0.71 \), the other with \( f_c = 51.1 \) Hz and \( k = 0.28 \). The curves for the separate components are shown as dashed lines in \( C \) and \( F \). Thus the complex random cross-correlograms seem to be resolvable into two components with properties appropriate for the chain and \( b_2 \) fibers, respectively. The second, slow peak does not appear to be due to rhythmic firing, because in Fig. 8 the correlograms for one of the gamma effects (A and B) are not appreciably different from those for the other (D and E), although the gain plots show clear evidence of rhythmicity in the bottom one but not in the top.

It was mentioned above that in some cases of correlograms with an apparent small second component, this could not be fitted with a second lognormal curve. In the gain plot transformations, however, the second component could be resolved by its additional contribution at low frequencies. This more effective and objective separation of the two components seemed to be a particular advantage of deriving the frequency domain version of the data.

The values for zero frequency gain (\( k \)) and corner frequency (\( f_c \)) are summarized in Table 2. Estimated separately, the mean \( k \) for fast components is twice that for slow components, but in mixtures the relative sizes are reversed. The other feature of note is that the mean \( f_c \) for the fast component measured in mixed effects was twice that found in pure chain effects. The reason for these observations is not clear, but as with the findings in Table 1, it is evident that some interactions do occur between the two fiber types in the initiation of impulses.

![FIG. 7. Comparison of cross- and autocorrelograms and gain plots for two pure \( b_2 \) effects. The layout is the same as in Fig. 6. A–C: weak \( b_2 \), effect with marked rhythmicity. D–F: stronger \( b_2 \), effect with weaker rhythmicity.](http://jn.physiology.org/)
Considerations of linearity

When a system is energized with a white noise input, the cross-correlogram of the input with the output provides an estimate of the linear part of the system impulse function that is orthogonal or independent of nonlinear components (Wiener 1958). Given that a Poisson-distributed stimulus train does in effect constitute a white noise input, it may be legitimate to regard the relevant cross-correlograms shown above as linear impulse functions. If this is so, the superposition principle for linear systems should apply. That this is the case seems to be supported by the fact that the correlograms from apparently mixed effects can be analyzed into the linear sums of fast and slow components as found separately for apparently pure chain and bag₂ effects, respectively. In the frequency domain also, the gain curves for mixed effects can be fitted by the sum of two first-order lag curves with corner frequencies expected from analyses of pure chain and bag₂ effects. As a further check, Fig. 9 shows the results of stimulating two static γ-axons that had mixed effects on one primary ending. Each unit was stimulated with an independent Poisson train with mean frequency of 25 Hz. In Fig. 9A the sum of the cross-correlograms obtained with the stimuli applied separately (○) is compared with the correlogram obtained with the stimuli applied together (●). Unnormalized counts are used here, rather than probabilities, to allow for summation. In Fig. 9B the equivalent gain plots are shown. It is evident that the linear prediction works well for the main features of the correlograms and for the gain plots up to ~30 Hz.

Definitive classification of the static effects

The arguments explained above have been taken to justify the use of the random stimulation method to classify the gamma effects as c, b₂, or b₂c according to the apparent involvement of chain or bag₂ intrafusal fibers. The final assessment yielded the numbers of gamma static effects observed in the different classes as follows: c, 16 (23.5%); b₂, 17 (25%); and b₂c, 35 (51.5%).

From the above data, taking the observations of the different effects as statistically independent, we can derive the probability of seeing a chain effect (P_c) as 0.750 and the probability of seeing a bag₂ effect (P_b) as 0.765. The theoretical probability of seeing a pure chain effect would be \( P_c(1 - P_b) = 0.177 \), and the probability of seeing a pure bag₂ effect would be \( P_b(1 - P_c) = 0.191 \). Thus the expected numbers would be as follows: c, 12; b₂, 13; and b₂c, 43 (the last number is derived as 68 – (12 + 13) because there can be no nil effects). Comparing the observed with the expected numbers gave a value for \( \chi^2 = 4.05 \), which for 1 degree of freedom would be exceeded by chance for 0.025 < \( P < 0.05 \). Thus there appears to be some statistical basis for rejecting the null hypothesis that the contacts of γ-axons to bag₂ and chain fibers are distributed independently. There are more pure chain and pure bag₂ contacts and less mixed contacts than expected.

Quantitative aspects of static effects

From the functional point of view, the qualitative division of gamma effects into the rigidly distinct types as above may be less important than consideration of the relative quantitative effects. Thus, if a group of γ-axons, which is not entirely specific, nevertheless affects one intrafusal fiber type more strongly than the other, then there will exist a possibility for the CNS output to be weighted toward one or the other. The method of random testing presented here in fact offers an opportunity of estimating the strength of each γ-axon’s effect on bag₂ and chain fibers through the estimates of gain from the appropriate best fit first-order lag curves. Figure 10 presents this data for the 60 gamma effects of 68 for which it was possible to measure the gain values. Gain could not be measured in five cases of weak pure bag₂ effects, two chain effects, and one bag₂ plus chain effect. The scatter plot shows that the pure bag₂ and chain effects have similar ranges of gain values, but that the mixed ones are predominantly weighted toward stronger bag₂ influence. Nonspecific distribution of γ-axons to bag₂ and chain fibers might have been expected to result in data points being scattered evenly about the diagonal line of equality, but this is evidently not so.

It could be argued that a perfectly pure effect on one or other of the two fiber types is an unnecessarily strict requirement for classifying the static γ-axons and that it may be more appropriate to identify the predominant effect. The mixed effects have therefore been reexamined, and where the strength of the effect on one fiber type (estimated from the gain plots) was more than five times greater that on the other, then it was reassigned to the stronger category. This moved 11 points from the mixed category to the bag₂ group and left the chain number unchanged. Taking this new distribution of effects, the \( \chi^2 \) test indicated that the deviation from the expectations of a nonspecific innervation was highly significant (\( P < 0.001 \)).

DISCUSSION

The objectives of this study were to examine a novel method for distinguishing the activation of bag₂ and chain intrafusal fibers, namely the use of Poisson-distributed gamma stimuli, and to use it to gather more evidence as to whether or not these two fiber types can be operated sepa-
The method depends, as have previous methods, on the different mechanical contraction properties of bag\textsubscript{2} and chain fibers. Boyd (1985) believed that, because of the fast-twitch characteristics of chain fibers, their activity could always be detected by signs of driving during the ramp frequency stimulation test. However, Dickson et al. (1993) pointed out that chain activation could be missed under some conditions by this test, even when it was made more sensitive by cross-correlating the Ia afferent firing with the stimulus train. More recently, Celichowski et al. (1994) have exploited the lower tetanic fusion frequency of bag\textsubscript{2} relative to chain fibers by comparing afferent responses during regular gamma stimulation at 30 and 100 Hz. Any static gamma effect that failed to cause a significant departure from a flat baseline in the cross-correlogram with 100-Hz stimulation was taken to be due to pure bag\textsubscript{2} activation. The disadvantage of this test is that, because of the regular 100-Hz stimulation, only a 10-ms length of cross-correlogram can be examined. This precludes any detailed examination of the form of the response and gives no direct evidence of any bag\textsubscript{2} contribution or any measure of the strength of the effects.

Identification of bag\textsubscript{2} and chain effects

The possibility of using a random, Poisson-distributed, stimulus train combined with cross-correlation to examine fusimotor effects has been suggested previously (Boyd and Rosenberg 1985; Homma and Nakajima 1985) and the advantage noted of showing a chain effect as a single peak on a flat baseline in the correlogram. We have indicated previously (Durbaba et al. 1993; Taylor et al. 1996), and now exploit in some detail, the fact that both chain and bag\textsubscript{2} effects can be detected and measured by using long runs of random stimuli. The development of this technique has been facilitated by the recognition that the fast and slow components in the correlogram can be fitted very effectively by means of lognormal curves. Although the lognormal curve is essentially an arbitrary choice, it may have some theoretical justification because it is closely approximated by the output of three or more cascaded low-pass filters energized by a pulse (Band et al. 1997). The lognormal form of the response may thus be a consequence of there being several stages of low-pass filtering in the transduction process from gamma impulse to afferent impulse. It has also been shown here that, by transforming the data into the frequency domain, gain plots may be constructed that can be generally well fitted by first-order lag curves. These in turn have provided sensitive and objective ways of detecting and measuring the strength of bag\textsubscript{2} and chain fiber effects. It is perhaps surprising that the responses to \( \gamma \) stimulation can be analyzed so effectively by linear methods and in particular that bag\textsubscript{2} and chain effects apparently sum linearly. This may be a consequence of there being a single impulse initiation site shared by the bag\textsubscript{2} and chain fibers at which the generator
Interpretation of the findings

There are two ways of examining the distribution of static axons to bag₂ and chain fibers for the purposes of looking for evidence that they might be controlled separately from the CNS. On the one hand, the distribution of single γ-axons to several spindles can be studied to see to what extent the innervation is specific for a particular fiber type. This is the approach used by Dickson et al. (1993) and Celichowski et al. (1994) in studying muscles containing only small numbers of spindles. On the other hand, when studying large muscles such as gastrocnemius, as in the present case, it is difficult to find γ-axons projecting to multiple spindles, even when recording from up to 12 afferents. In this situation the only possibility is to examine the relative proportions of static effects that can be diagnosed as pure bag₂, pure chain, or mixed and to compare them with the statistical expectation on the assumption that contacts with the two types are independently distributed. The present findings provide some statistical evidence in favor of a degree of specificity in that the observed distribution would only be expected by chance with a probability of between 2 and 5%. In the study by Celichowski et al. (1994) using the much smaller muscle peroneus tertius, it was possible to look at the effects of single γ-axons on three or four spindles. The criterion for specificity in that work was, however, very strict in that it emphasized the requirement that a given γ-axon should have the same action on all afferents affected. We have now taken the whole published data in Celichowski et al. (1994), including those effects seen on only one or two afferents and have recalculated probabilities according to the method used in the present work. Under these conditions the peroneus tertius data also show a significant departure from the expectations of a nonspecific distribution. It will be useful to gather more data of this kind to explore the relationship between the two alternative views of specificity. It will also be important to examine different muscles, in case the innervation patterns differ according to some different functional requirements.

The above considerations were based purely on the qualitative presence or absence of contacts of given γ-axons on bag₂ and chain fibers. The random correlation method as described here provides additionally the opportunity of taking into account the strength of the connections, so that it is possible to redefine the “pure” bag₂ and chain categories instead as predominantly bag₂ or predominantly chain, when the gain of one component is at least five times that of the other. With the use of this approach, the present data indicate a much more highly significant departure from the expectations of a nonspecific innervation. The case for there being some difference between the gamma motoneurons innervating bag₂ and chain is supported in a different way by the CV measurements. We observed that the mean CV for γ-axons supplying purely bag₂ fibers was significantly higher than for those supplying chain fibers, a finding similar to that of Dickson et al. (1993).

Functional implications

The effects of bag₂ fiber contraction on the responses of Ia afferents to muscle stretch are quite clear. Tonic stimulation has a strong biasing effect, which leaves the response to muscle stretch largely unaffected, especially preserving the resonance effects that result from mixing regular stimulation with the natural rhythmicity of the afferent. The latter emerges as a peak in the gain plot centered on the natural firing frequency.

The justification for a purely physiological test such as this in the absence of direct visualization of the intrafusal contraction (Dickson et al. 1993) is inevitably indirect. However, the interpretations arrived at here are thought to be valid because of their consistency internally and with those of the previously accepted methods. One problem that remains is the possibility that a particularly strong bag₂ effect [perhaps associated with a bag₂ fiber supporting a propagated action potential as suggested by Dickson et al. (1993)] might produce a correlogram peak large enough to be confused with a chain fiber effect. It is felt that this possibility is eliminated in the present work by the recognition that the corner frequency of the gain plot in this case would have a low value (∼3–9 Hz) in contrast to the higher values (30–140 Hz) characteristic of chain effects.
sufficient to allow the CNS reasonable independence in the control of the spindle properties that, as indicated above, depend on their separate action. To answer this question, it will be necessary to look at the effects on spindle properties of combining various proportions of bag₂ and chain activation. This might be attempted in the future by systematically examining the changes in stretch properties associated with the various gamma effects ranging from pure bag₂ to pure chain.

One reasonable scheme based on these principles for using the gamma system during some natural movements would therefore be as follows. At the beginning of a movement sequence dynamic fusimotor activity and bag₂ static fusimotor activity would be increased tonically to set appropriate sensitivity and bias [‘‘fusimotor set’’; reviewed by Prochazka (1996)]. When the active shortening occurred, the γ-motoneurons supplying bag₂ and chain fibers might be activated approximately in parallel with the α-motoneurons to help offset the unloading of the spindles, which could therefore continue to monitor the movement and in some cases provide for load compensation through ‘‘servo-assistance’’.

This scheme represents a synthesis of various previous ideas (see Matthews 1972; Taylor and Appenteng 1981; Taylor et al. 1995b) now extended to incorporate the evidence that there is some degree of specificity in the innervation of bag₂ and chain intrafusal fibers by the static gamma motor system. The specificity of control clearly can only be partial, but in principle there is no need for it to be complete for the CNS to adapt to use it to best advantage.

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