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ORGANIZATION OF INPUT TO THE INTERNEURONES MEDIATING GROUP I NON-RECIPROCAL INHIBITION OF MOTONEURONES IN THE CAT

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WITH AN APPENDIX BY P. J. HARRISON

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SUMMARY

1. Patterns of convergence of different presynaptic fibre types onto interneurones mediating non-reciprocal inhibition of motoneurones have been studied in order to investigate to what extent the population of these interneurones is homogeneous or can be divided into subgroups on the basis of their input.

2. In a sample of interneurones, all of which were interposed in pathways from the group I afferents of one group of muscles (triceps surae and plantaris), individual interneurones exhibited a wide variety of convergence patterns. Some interneurones were influenced by only a few types of afferent or descending fibre systems whereas others were influenced by many. Furthermore, various fibre systems excited and/or inhibited individual interneurones in different combinations.

3. While there appeared to be too many patterns of convergence to allow any simple classification into a few distinct groups of interneurones, two possibilities were considered. One was that certain presynaptic fibre types influence individual interneurones in preferred combinations. The other was that they converge entirely at random. To investigate this, the frequencies of convergence of various pairs of fibre types were predicted assuming that each of them influences a proportion of the interneurones independently of other sources. Generally, there was close correspondence between such predicted and observed frequencies of occurrence of tested combinations of input. These findings are thus compatible with an organization whereby individual presynaptic fibres innervate a random sample of the population of interneurones.

4. Deviations from the predicted incidence of convergence patterns were found primarily for synaptic actions mediated di- or oligosynaptically and are attributed to a consequence of convergence at the pre-interneuronal level.

5. A particular consequence of such an organization is that interneurones in pathways of non-reciprocal inhibition are shared by afferents of different muscles in a continuum of combinations. The functional implications of this arrangement are discussed.

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INTRODUCTION

The results presented in the preceding paper (Harrison & Jankowska, 1985) have shown that various fibre types influence only a proportion of the population of interneurones mediating group I non-reciprocal inhibition. In a further analysis of the input to this population, we have analysed patterns of convergence onto individual interneurones to see whether they fall into distinct groups on the basis of these patterns. We considered that certain fibre types might converge onto one group of interneurones and other fibres onto other groups. For example, transmission through some interneurones might be under predominantly descending control and through others under control from say cutaneous or joint afferents; or different groups of interneurones may be associated with the activity of different muscles. This clearly has implications for the various theories regarding the reflex actions of group Ib afferents and for the organization of the population of these interneurones in general.

METHODS

The data used in the analysis of input to the interneurones mediating non-reciprocal inhibition, are drawn primarily from the material presented in the accompanying paper (Harrison & Jankowska, 1985) to which the reader is referred for all experimental details.

Because of the nature of the analysis, it was desirable to consider the input from as many presynaptic sources, and in as large a sample of neurones, as possible. The analysis has therefore been limited to the most extensively studied synaptic actions, i.e. those from group I afferents (from six muscle nerves), cutaneous afferents (from two nerves), posterior knee joint afferents, afferents travelling in the interosseous nerve, the rubrospinal tract and the corticospinal tract. The distribution of excitation and inhibition from these sources was studied in a total of eighty-six interneurones. Seventy-four of these had group I input from triceps surae and/or plantaris and have been described in the companion paper. Twelve supplementary interneurones fulfilled the same criteria of interneurones mediating non-reciprocal inhibition of motoneurones but were excited by group I afferents of muscles other than triceps surae and plantaris.

RESULTS

Do interneurones mediating non-reciprocal inhibition fall into distinct subgroups on the basis of their patterns of convergence?

For a variety of reasons, it was frequently the case that synaptic actions tested in individual interneurones did not include actions from all the presynaptic fibre systems. The effects of stimulation of eleven systems (all tested in each interneurone) were investigated in eighteen interneurones and Fig. 1 shows the patterns of convergence upon individual interneurones. These were excited by group I afferents of one to five muscle nerves and by none to four other fibre systems (Fig. 1*A*). The variability of inhibitory input was similar (Fig. 1*B*). Of the eighteen interneurones, only two (numbers 10 and 11) showed the same excitatory input but they differed with respect to the inhibitory input. Similarly, only two pairs of interneurones (numbers 4 and 10, and 5 and 18) showed the same inhibitory input, though they differed with respect to the excitatory input. Generally, both these and other interneurones showed patterns of convergence with too many combinations to allow any simple classification into a few distinct groups.

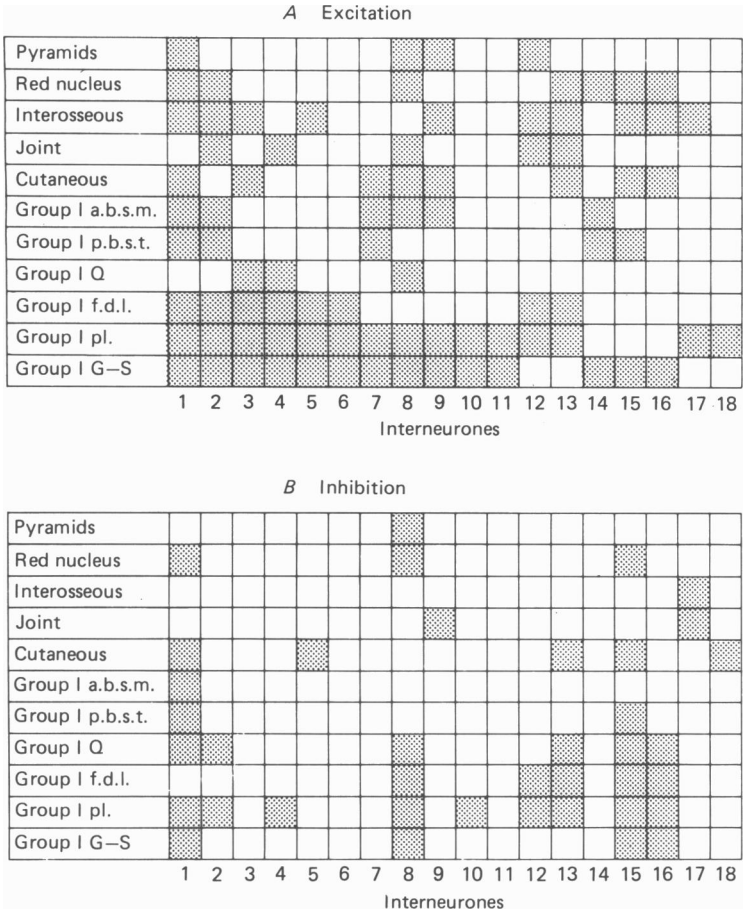

Fig. 1. Patterns of convergence exhibited by the most extensively tested group of eighteen interneurones. The data for individual interneurones are in the vertical columns which are marked with successive numbers, valid for both graphs. The tested fibre types are indicated to the left, the shading showing which of them evoked excitation (*A*) and/or inhibition (*B*) of a given neurone. Of the various synaptic actions only those evoked via the most direct pathways (i.e. mono-, di- or trisynaptic for excitation, and di- or trisynaptic for inhibition) are considered in this diagram. The criteria for classification of the p.s.p.s were as described in Harrison & Jankowska (1985). The interneurones have been ranked from those with excitatory input from the largest to the smallest number of muscle nerves of ankle and toe extensors, independently of the order in which they were recorded. With respect to the negative data, it should be noted that all the fibre systems were tested in each interneurone and that they were effective in exciting and/or inhibiting other interneurones in the same experiments. Abbreviations: a.b.s.m., anterior biceps and semimembranosus; p.b.s.t., posterior biceps and semitendinosus; Q, quadriceps; f.d.l., flexor digitorum longus; pl., plantaris; G–S, gastrocnemius–soleus.

Is the input from different types of fibre associated in any way?

In view of the wide variety of convergence patterns, we had to resort to a statistical approach to see if there were any patterns that were not obvious by eye. With this approach we considered the distribution of input from pairs of different sources to find out if at least some presynaptic fibre types influence individual interneurones

in preferred combinations. One premise was that if two presynaptic fibre types tend to converge on the same interneurones, then the observed frequency of joint occurrence (or lack of occurrence) of p.s.p.s evoked by both of them should be greater than the frequency expected on a random basis. A comparison of the two frequencies should therefore reveal any association in input from different sources.

If the different fibre types were to evoke post-synaptic potentials (p.s.p.s) on a random basis, then the probability of combinations of these p.s.p.s can be calculated. Thus, consider input from fibre types A and B, which evoke p.s.p.s in 40 and 60% of the interneurones respectively. We would expect to find 24% excited by both A and B, 16% excited by A only, 36% excited by B only and 24% excited by neither A nor B. (The probability of both occurring $= A \times B$; the probability of the first only occurring $= A(1-B)$; the probability of the second only occurring $= B(1-A)$; the probability of neither occuring $= (1-A)(1-B)$; see, e.g. Tranter & Lambe, 1970.) Such figures were obtained for the different combinations of input. These were then compared with the actual frequencies of occurrence of excitatory post-synaptic potentials (e.p.s.p.s) or inhibitory post-synaptic potentials (i.p.s.p.s) found in the interneurones in which the two sources of input were tested.

Fig. 2*A* shows results of such an analysis for e.p.s.p.s evoked by group I afferents, each histogram being for a different pair of muscle nerves. For example, the uppermost histogram is for intereurones in which plantaris and gastrocnemius–soleus group I afferents were tested. The four *open* columns of this histogram indicate, from left to right, the *observed* percentage of interneurones excited by both gastrocnemius–soleus and plantaris, the percentage of interneurones excited by plantaris only, the percentage of interneurones excited by gastrocnemius–soleus only and the percentage of interneurones excited by neither. The *filled* columns give the *calculated* percentages of interneurones for the same combinations assumed to occur on a random basis. The latter will be referred to in the following as 'predicted' percentages.

In Fig. 2*B* are similar histograms for pairs of non-muscular sources of input. Both sets of data show that, in general, there is quite close correspondence between the observed and the predicted percentages. Thus, they indicate that, to a first-order approximation, the patterns of convergence onto these interneurones can be explained on the basis that different presynaptic fibres connect randomly with the population of interneurones. Similar results were found when considering the joint occurrence of e.p.s.p.s from various group I muscle afferents and from other sources of input (e.g. plantaris *vs.* red nucleus; plantaris *vs.* pyramid; quadriceps *vs.* red nucleus).

The histograms of Fig. 2 are for all the interneurones, independently of the origin of their group I input, and for mono- as well as di- and trisynaptic pathways. By lumping all the data together in this way any gross associations of input should be revealed. Since no such associations are apparent, these data have also been subdivided in different ways to see whether any associations of input could then be found. Thus, we divided the whole population into subgroups and considered mono- and di- and trisynaptic pathways separately. For convenience of presentation, the data of the two left-hand columns (and only the left-hand columns) of histograms such as in Fig. 2 were used for this purpose. These data are shown as plots of 'percentage of interneurones observed to be co-excited by various pairs of presynaptic fibre types' against 'percentage of interneurones predicted on a random basis'

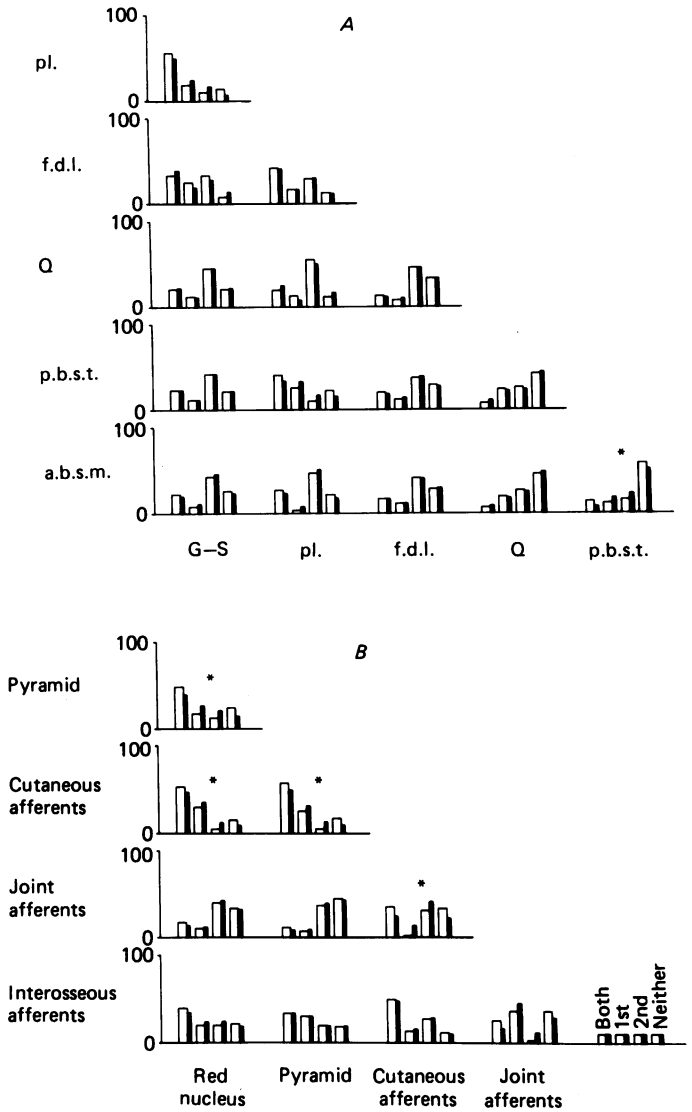

Fig. 2. Histograms of observed and predicted (on a random basis) distribution of input from pairs of excitatory presynaptic fibre types. The columns are from the left to the right for co-excitation by both types of fibre, excitation from the first (ordinate) only, second (abscissa) only or neither, as indicated in the inset. Open columns: observed proportions of the co-excited interneurones. Filled columns: predicted probability of occurrence of co-excitation. *A*, comparison of input for different group I muscle afferents, indicated to the left and below. *B*, similar comparison considering excitation from non-muscular sources of input. Asterisks indicate statistically significant differences ($P < 0{\cdot}01$, χ^2 test). The illustrated data are for all interneurones and for all mono- and oligosynaptic connexions lumped together. Abbreviations, as in Fig. 1. For further explanations see text.

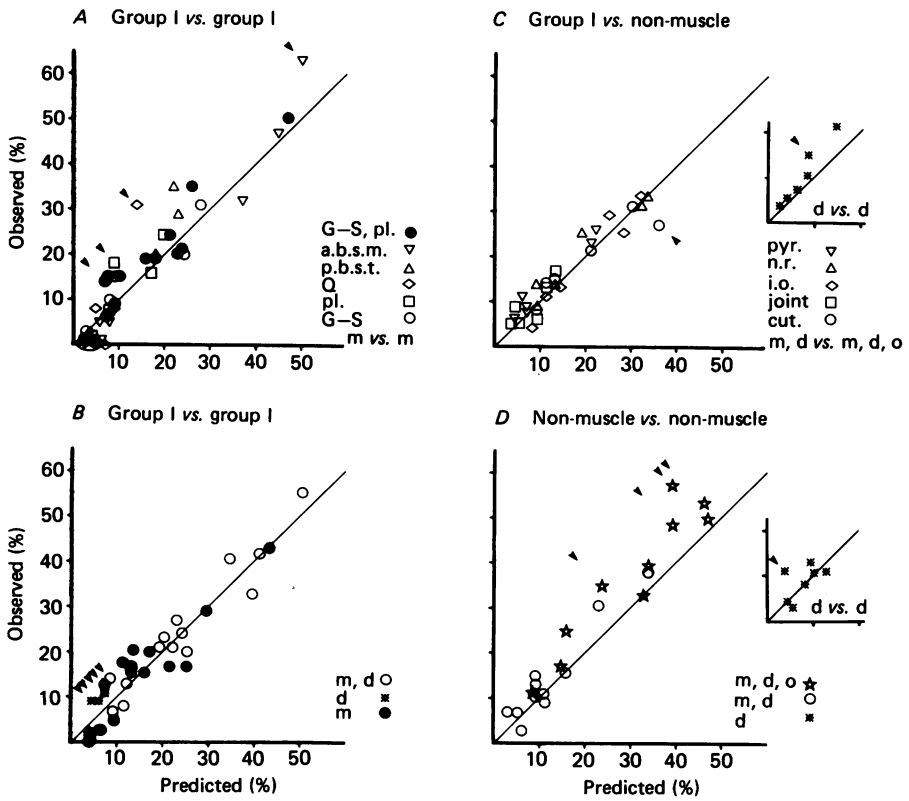

Fig. 3. Plots of observed percentages of interneurones excited by both of two tested fibre types *vs.* those predicted on a random basis. *A*, for interneurones with monosynaptic input from one particular muscle (indicated by different symbols), percentage of interneurones co-excited monosynaptically by group I afferents of other muscles taken in pairs. *B*, comparison of input from group I afferents via oligosynaptic pathways including both mono- and disynaptic ones, or via only monosynaptic (m) or disynaptic (d) pathways in the whole sample of interneurones. *C*, similar comparison for pairs of various group I afferents and various non-muscular types of fibres (which are indicated by the different symbols). Lumped data for mono- and disynaptic pathways from group I afferents and for any oligosynaptic (o) pathways including mono-, di- and trisynaptic ones, of other fibre types. Of these, data for pairs of disynaptic pathways are plotted in the inset. *D*, similar comparison for pairs of non-muscular fibre types, with different symbols for actions evoked via different categories of pathways, as indicated. Data for disynaptic pathways only are plotted in the inset. The statistically significant differences between the observed and predicted frequencies are indicated by arrowheads. These were for the following combinations: in *A* for: G–S,pl. (▽◇) and p.b.s.t.–a.b.s.m. (●□). In *B* for: p.b.s.t.–a.b.s.m. (●○), and G–S–pl., and G–S–Q, pl.–Q (*). In *C* for: pl.–cut. (○★). In *D* for: n.r.–pyr. (*), and for cut.–pyr., cut.–n.r., cut.–joint, n.r.–pyr. (☆). Cut., cutaneous; pyr., pyramids; n.r., red nucleus; i.o., interosseous.

(Fig. 3). Most of the data points of all these diagrams are distributed along or close to the continuous lines which represent the relation expected if there was strict correspondence between the observed and predicted convergence patterns. Any statistically significant differences are indicated by arrow heads ($P < 0{\cdot}01$; χ^2 test).

The plots of Fig. 3*A* are for the joint occurrence of monosynaptic e.p.s.p.s evoked

from six muscle nerves, considering two nerves at a time. For interneurones with monosynaptic group I input from both gastrocnemius–soleus and plantaris (as in the companion paper), patterns of convergence of monosynaptic group I input from other muscle nerves were of practically the same distribution as those predicted on a random basis (Fig. 3*A*, ●). Similarly, considering only interneurones with monosynaptic input from any particular muscle (indicated by different open symbols in Fig. 3*A*), patterns of convergence from the remaining muscle nerves showed similar

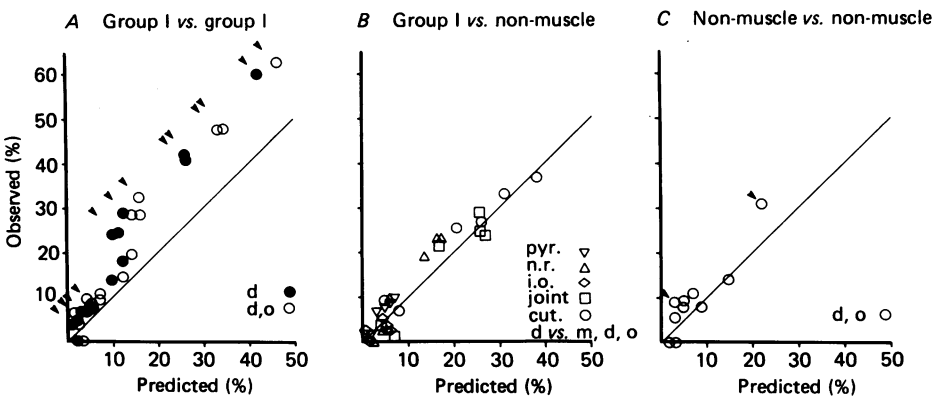

Fig. 4. Plots of observed proportions of interneurones inhibited by both of two tested fibre types *vs.* those predicted on a random basis. *A*, comparison for disynaptic only or for oligosynaptic (o) including both di- and trisynaptic inhibition from various group I afferents. *B*, comparison for pairs of group I afferents and non-muscular types of afferents. *C*, comparison for pairs of non-muscular fibre types. The statistically significant differences (arrowheads) in *A* are for all combinations involving pl.–G–S, pl.–f.d.l., pl.–Q, p.b.s.t.–a.b.s.m. and Q–a.b.s.m. and for disynaptically evoked inhibition from Q–f.d.l. The differences in *C* are for combinations of joint and interosseous afferents and for joint and cutaneous afferents. Abbreviations as in Figs. 1 and 3.

results. Of the thirty combinations only four showed statistically significant differences. Considering that there are topographical factors tending to bias the patterns of convergence (see Discussion) it appears as if group I afferents of any particular muscle generally excite a random proportion of the whole population of interneurones mediating group I non-reciprocal inhibition. Consequently, for the purposes of the following analysis interneurones with group I input from various muscles will be considered together as one group.

In Fig. 3*B* the patterns of convergence for all the interneurones are plotted for different categories of excitation, i.e. for excitation evoked monosynaptically (●), disynaptically (*) and either mono- or disynaptically (○). These plots show the same correspondence between the observed and predicted patterns of convergence. However, statistical analyses show that the incidence of joint occurrence of disynaptic excitation is significantly greater than that predicted for all three combinations of disynaptic excitation, while only one such statistically significant difference occurred for the monosynaptic connexions, or when the mono- and disynaptic connexions were lumped together. (Note that different samples of data were used for the plots in Fig. 3*A* and Fig. 3*B*, which are therefore not inconsistent.)

Plots of Fig. 3*C* show good correspondence between the observed and predicted frequencies of joint actions of group I afferents and of non-muscular fibre types for all but two combinations. When the data for I a and I b subgroups of group I afferents were separately considered the results were similar. In contrast, data points for pairs of non-muscular types of input (Fig. 3*D*) showed a consistent tendency to lie on one side of the continuous line and several differences were statistically significant, particularly when di- or trisynaptic pathways were involved.

In the cases of inhibitory actions, a similar comparison has revealed a close correspondence between the observed and the predicted convergence patterns for the combinations shown in Fig. 4*B* and *C*; there were no statistically significant differences for pairs of group I and non-muscular sources of inhibition (*B*) and there were only two such differences for pairs of non-muscular sources of inhibition (*C*). The joint inhibitory actions from group I afferents were on the other hand seen more freqently than predicted on a random basis (Fig. 4*A*; see, however, Discussion and Appendix).

DISCUSSION

The results of the companion paper (Harrison & Jankowska, 1985) and of the present analysis show not only that the interneurones mediating non-reciprocal inhibition exhibit a high degree of convergence from several types of primary afferents and/or descending fibres, but also that the number of patterns of convergence is very large and that these interneurones do not fall into major subgroups (like those considered in the Introduction) on the basis of their input. Furthermore, by predicting the frequency of coincidence of synaptic actions evoked by pairs of afferent fibre types and comparing such predicted frequencies with those actually observed, our results indicate that the patterns of convergence are largely explicable on the basis that different presynaptic fibres connect independently of each other within the population of interneurones.

Randomness and topographic factors in the formation of synaptic connexions

The factors which determine input onto a given population of neurones intermixed with neurones of other types, as in the case of the investigated population of interneurones in the intermediate zone of the spinal cord, are poorly understood. however, among these factors must be those deciding about *specific connexions to functionally different neurones* and those involved in *the random formation of synaptic contacts upon neurones of the same population*. With respect to the latter, it is becoming clear that the probability of formation of synaptic contacts of I a afferents within a given motoneurone pool is highly dependent upon the density of terminal aborizations near a particular motoneurone and that I a afferent fibres tend to make synaptic contacts with a higher proportion of motoneurones in the centre rather than on the outskirts of their termination area (see Scott & Mendell, 1976; Lüscher, Ruenzel & Henneman, 1980; Lüscher, Mathis & Henneman, 1984). One may therefore assume that the probability of formation of synaptic contacts of the various afferent fibre types on the interneurones mediating non-reciprocal inhibition would similarly be a function of the density of terminals of these fibres in laminae V–VI. In this respect,

it is important to note that these interneurones are distributed over a length of spinal cord at least twice as long as that of their target motor nuclei (i.e. over four or five segments or some thirty millimetres; Hongo, Jankowska, Ohno, Sasaki, Yamashita & Yoshida, 1983; see also Jankowska, Johannisson & Lipski, 1981). The considerable length of the column of these interneurones might be a requirement for interneurones to be contacted by afferents entering, and branching, at different segmental levels. A location within one of the more rostral or more caudal segments may accordingly be one of the factors influencing the probability that individual interneurones will be contacted by various fibres.

Location within a more dorsal or more ventral part of the intermediate zone might be another factor, as is indicated, for example, by the observation that a much larger proportion of the investigated interneurones exhibited monosynaptic e.p.s.p.s from rubrospinal than from corticospinal tract fibres, the terminal area of the rubrospinal fibres overlapping to a greater extent (cf. Nyberg-Hansén, 1966) with the area of location of these interneurones. With respect to the input from group I afferents, it has been reported that the terminal areas of I b afferents are wider than of I a afferents in the transverse plane (Brown & Fyffe, 1978, 1979; Brown, 1981; Hongo, Ishizuka, Mannen & Sasaki, 1978; Ishizuka, Mannen, Hongo & Sasaki, 1979; see also Eccles, Fatt, Landgren & Winsbury, 1954, for the distribution of monosynaptic field potentials). The larger I b terminal areas may thus be related to a selective input from I b but not I a afferents of ankle and toe extensors to some laminae V–VI interneurones (Jankowska *et al.* 1981; Harrison & Jankowska, 1985). The existence of a larger number of terminals per I b collateral than per I a collateral (cf. Brown & Fyffe, 1979; Brown, 1981; Hongo *et al.* 1978; Ishizuka *et al.* 1979) in laminae V–VI may likewise contribute to the larger number of interneurones with I b than with I a input as well as to the generally stronger excitation of interneurones in this area by I b than by I a afferents. Furthermore, a larger number of terminals per collateral of I a afferents of medial gastrocnemius, soleus and plantaris, than of hamstring I a afferents (Ishizuka *et al.* 1979) would similarly be reflected in the relative contribution of these afferents to the excitation of interneurones in this area. Only the high density of terminals of I a afferents of flexor digitorum longus has no equivalent in the relatively infrequent actions from this nerve on the investigated neurones.

The location of an interneurone within the centre of dense overlapping termination areas of two types of fibre might greatly increase the probability of its co-excitation by these fibres and thereby favour certain convergence patterns. Such a situation is indeed expected to occur for interneurones with input from group I afferents of gastrocnemius–soleus, plantaris, flexor digitorum longus and quadriceps in view of the dense overlapping projections of these afferents within some areas but not others (cf. Ishizuka *et al.* 1979). The fact that the investigated interneurones were most often tracked for within the areas of maximum field potentials from these nerves would further increase the probability of finding those co-excited. For these reasons we do not consider that the deviations from the 'predicted' frequencies, particularly in Fig. 3*A*, contradict the general conclusion that group I afferents of different muscles influence the investigated population of interneurones independently of each other.

One of the consequences of this general conclusion is that group I afferents of different muscles excite not only a random proportion of interneurones, but also act

in random combinations with group I afferents of other muscles. This in turn, is of prime importance for evaluating the various hypotheses regarding the functional role of reflexes from I b afferents, since input from several muscles in random combinations upon interneurones which mediate these reflexes precludes the possibility of primarily autogenetic feed-back, unless there were some highly specialized presynaptic control (see also Harrison, Jankowska & Johannisson, 1983; Harrison, 1985).

With respect to the excitation or inhibition of interneurones evoked via di- or trisynaptic pathways, a close correspondence between our observed and predicted patterns would be expected to come about primarily if the different presynaptic fibre types operated through different first-order interneurones (as shown in the Appendix), and the case of Fig. 4*B* might represent such a case. However, this would mean that inhibition from non-muscular afferent fibres (when stimulated alone) would be mediated mainly by interneurones other than those which evoke inhibition of group I origin, and would to only a relatively small extent be the result of mutual inhibition between the latter (Harrison & Jankowska, 1985; see Brink, Jankowska, McCrea & Skoog, 1983). Since group I and other types of fibre converge upon the same interneurones, this would further mean that the actions of non-muscular afferent fibres are often too weak to discharge these interneurones by themselves.

Deviations from the 'predicted' frequencies of co-excitation or co-inhibition evoked via di- and trisynaptic pathways might be explained in several non-exclusive ways. They might reflect (i) preferred patterns of input upon the first-order interneurones, or (ii) preferred connexions between the first and last-order interneurones, or (iii) an entirely random connectivity, as described in the Appendix. Preferred connexions could be useful in assisting in the selection of only some interneurones in the control of movement and in the shaping of different motor synergies (see Hongo, Jankowska & Lundberg, 1969, 1972). The existence of such connexions has in particular been postulated when the role of mutual inhibitory interactions between subgroups of interneurones has been discussed (Brink *et al.* 1983; Jankowska, 1982–83). However, we as yet lack information which could allow us to evaluate the relative contribution of any preferred connexions in oligosynaptic pathways to these interneurones. We will therefore only point out that if there were an entirely random connectivity in these pathways, one would in fact expect deviations from our 'predicted' frequencies of convergence (see the Appendix). Conseqently, the deviations from the 'predicted' convergence frequencies of Figs. 3*D* and 4 would be compatible with such a random connectivity.

'Fractionation' of input to the population of interneurones

If different presynaptic fibres influence interneurones of the present population entirely at random, it follows that the number of possible convergence patterns is 2^n; where n is the number of different sources of input considered. Taking the input from six different muscle groups would give sixty-four patterns if the I a and I b afferents were lumped together, and 4096 patterns if they were considered separately. Taking these together with the five non-muscular presynaptic sources considered in this study, would give 131 072 different combinations, with corresponding increases if the

different mono-, di- and trisynaptic linkages were considered separately. Since not all presynaptic sources have been considered in this analysis, the total number of possible patterns of convergence is clearly enormous!

Since the total number of interneurones mediating group I non-reciprocal inhibition (from any muscle afferents and to any motoneurone species) may be of the order of a few hundred, it is apparent that only some convergence patterns can be represented in the population and that each of these patterns may be expected in only a very small number of neurones. Consequently, it is not surprising that practically each one of the investigated neurones had a different pattern of input and that the 'fractionation' of input to these neurones is of the 'fine grain' type.

Such an organization may have several advantages. First, it might smooth differences in the input to individual interneurones of the population and secure a statistically uniform input to motoneurones onto which they terminate. If fifty or more interneurones inhibit individual motoneurones, they might include interneurones with so many of the input combinations that impulses from all the relevant fibre systems could be used for modulating the excitability of individual motoneurones. Such a smoothing may nevertheless be more effective for populations than for individual motoneurones, since not all the motoneurones of a homogeneous population are inhibited by the group I afferents of a particular muscle origin (Jankowska & Zytnicki, 1985).

Input fractionation might also serve other purposes. If all the fibres supplying input to a population of interneurones terminated on each individual interneurone, it is conceivable that these interneurones might be too easily excited, especially if impulses in two or three of these fibre systems sufficed for their discharge. Consequently, the possible range of gradation of the activity of these interneurones would be very limited. Still another use of input fractionation might be to allow any two or more fibre systems, activated in different combinations, to select a group of interneurones which could assist them in inhibiting a certain combination of motoneurones and thus secure a proper movement adjustment (Hongo *et al.* 1969, 1972; Brink *et al.* 1983; Jankowska, 1982–83). To serve this purpose, interneurones with different patterns of convergence should terminate on different motoneurones but, so far, we have only very limited information in this respect. For instance it appears as if interneurones with input from both proximal and distal muscles, or from distal muscles only, tend to project to different motor nuclei (see Fig. 10 in Jankowska *et al.* 1981 and Fig. 6 in Czarkowska, Jankowska & Sybirska, 1981, for unidentified laminae V–VI interneurones). On the other hand, at least some interneurones with input from any pairs of muscles appear to be involved in non-reciprocal inhibition of any tested species of motoneurones (Harrison *et al.* 1983). Clearly, future studies are needed to find out how the specific and chance factors combine in bringing about purposeful operation of the interneuronal systems and how they contribute to the organization of movements.

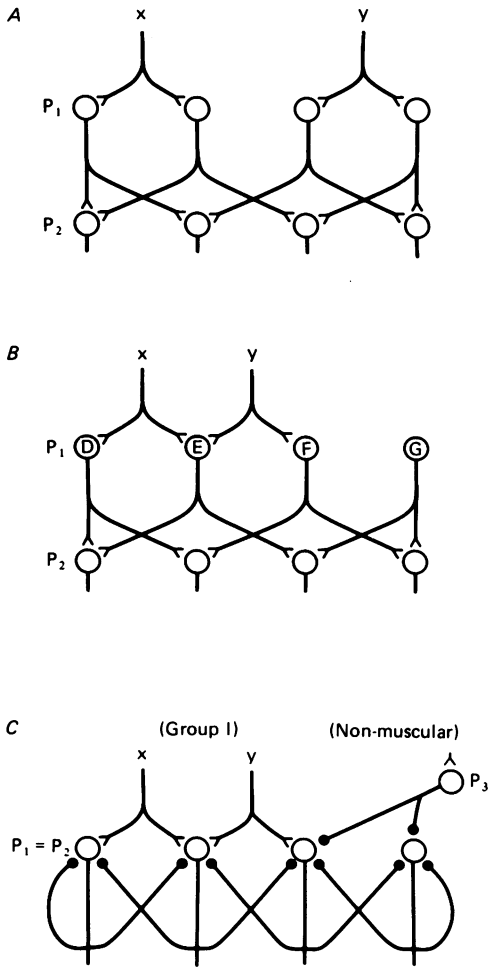

Fig. 5. Schematic diagrams of neuronal connexions in disynaptic pathways. In *A* and *B*, afferent fibres x and y innervate a population P_1 of interneurones. These interneurones have random connexions with the second order interneurones P_2. In *A* afferent fibres x and y innervate separate interneurones. In *B*, afferent fibres connect randomly with the population of interneurones P_1, hence some interneurones will be excited by both x and y. *C*, as for *B* but modified to account for organization of the inhibitory interneurones mediating group I non-reciprocal inhibition. Instead of connecting onto another population of interneurones, the axonal collaterals innervate (assumedly at random) the same population ($P_1 = P_2$) of interneurones. Non-muscular input is shown to inhibit these interneurones via a separate group of inhibitory interneurones (P_3).

APPENDIX

By P. J. Harrison

Some implications of the organization of input onto the first-order interneurones in disynaptic pathways

The aim of this Appendix is to illustrate how differences in the organization of input onto the first-order interneurones could affect the probability of co-excitation or co-inhibition of interneurones influenced disynaptically.

If a group of afferents (x or y in Fig. 5*A*) evokes discharges in a proportion (A) of a population (P_1) of N interneurones, then AN interneurones will discharge. If each interneurone excites a proportion (B) of a second population (P_2) of interneurones (also of size N), then the proportion of the second-order interneurones *not* excited will be $(1-B)^{AN}$. Therefore,

$$\text{the total proportion excited } (C_1) = 1-(1-B)^{AN}. \tag{1}$$

Thus, assuming that interneurones of population P_1 are connected randomly to population P_2 and that both x and y evoke discharges in equal proportions (A) of the first-order interneurones (P_1), the probability of any second-order interneurone of population P_2 being excited disynaptically by the group of afferents x or y, will also be $= 1-(1-B)^{AN}$. The probability of an interneurone of population P_2 being excited by *both* x and y will be the product of the two probabilities of excitation evoked independently, i.e.

$$C_2 = (1-(1-B)^{AN}) \times (1-(1-B)^{AN}). \tag{2}$$

This, however, also assumes that afferents x and y excite *separate* interneurones of the population P_1. If, however, the two groups of afferents (x and y) were to distribute randomly on the population P_1, then the following will show that the proportion of interneurones (of population P_2) excited by both x and y would be somewhat different.

If x and y each discharge a *random* proportion (A) of the population P_1, then any interneurones discharged in common by both x and y (labelled E in Fig. 5*B*) will evoke excitation in all second-order interneurones to which group E project. If x and y both discharge equal proportions (A) of the interneurones, then group E will total A^2N interneurones. Therefore, as for eqn. (1), the proportion of the population P_2 excited by both x and y, via group E, will be $= 1-(1-B)^{A^2N}$.

Of the interneurones of population P_2 not already excited by both x and y (via group E), i.e. $(1-B)^{A^2N}$, some will be excited by both x and y as a result of D and F converging onto interneurones of population P_2. Since x and y both discharge AN interneurones and group E totals A^2N interneurones, groups D and F will total $(A-A^2)\ N$ interneurones each. Thus, the proportion of interneurones excited by groups D and F will be $= 1-(1-B)^{(A-A^2)\,N}$.

Therefore, the proportion of interneurones excited by both x and y, via groups D and F (though not including those already excited via group E) will be:

$$[1-(1-B)^{(A-A^2)\,N}][1-(1-B)^{(A-A^2)\,N}][(1-B)^{A^2N}].$$

The total proportion of P_2 excited by both x and y, via groups D, E and F will therefore be:

$$[1-(1-B)^{(A-A^2)N}]^2[(1-B)^{A^2N}]+1-(1-B)^{A^2N}.$$

Rearranging, the total proportion of P_2 excited will be:

$$C_3 = (1-B)^{(2A-A^2)N}-2(1-B)^{AN}+1. \quad (3)$$

Since eqns. (2) and (3) are different, it follows that the joint occurrence of disynaptic excitation evoked by two groups of afferents will not necessarily be determined by the product of the probabilities of evoking excitation separately, but will depend at least partly on the organization of input to the first-order interneurones. Thus, the 'predicted' occurrence of convergence for di- and trisynaptic pathways in Figs. 2,

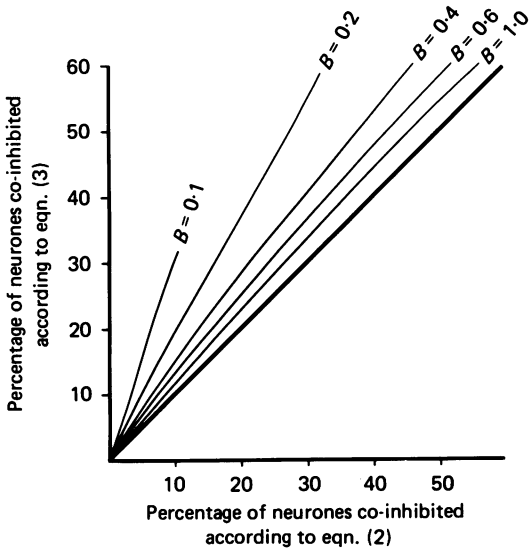

Fig. 6. Plots of percentages of neurones co-inhibited by two groups of afferent fibres calculated assuming random connectivity of primary afferents onto the first-order interneurones (eqn. (3) in text) against percentages calculated for two groups of primary afferents innervating separate first-order interneurones (eqn. (2) in text). The connectivity of the first-order interneurones to the second-order interneurones is assumed to be random. The value of N (the number of interneurones in each population of interneurones) is 400 for all curves. B signifies the percentage of second-order interneurones that each first-order interneurone projects to.

3 and 4 presupposes an interneuronal organization such as in Fig. 5*A*. On the other hand, an organization such as in Fig. 5*B* may lead to deviations from our 'predicted' occurrence of convergence. In order to simulate the extent to which such deviations will occur, 'predicted' frequencies of convergence can be calculated according to eqn. (2) and plotted against frequencies of convergence that would occur if the interneuronal organization were as in Fig. 5*B*, i.e. according to eqn. (3).

Since the problem is particularly applicable to inhibition of the laminae V–VI interneurones evoked via their recurrent collaterals, the problem will be discussed with this in mind. (In this case the population of P_1 is the same as population P_2.)

For any given population of interneurones, B and N are constant. The proportion of interneurones inhibited will therefore depend exclusively on the value of A, the proportion of interneurones discharged in response to x or y. For the population of interneurones mediating non-reciprocal inhibition, N can be approximated at about 400 (see especially Hongo *et al.* 1983). The value of B on the other hand, can be estimated from eqn. (1). Given that gastrocnemius–soleus or plantaris group I afferents inhibit about 60 % of the interneurones, that there are of the order of 400 interneurones mediating non-reciprocal inhibition, and that a sizeable proportion of the interneurones discharge in response to group I afferents of gastrocnemius–soleus or plantaris (say 60 %), the value of B is approximately 0·4 %.

Fig. 6 shows plots of the percentage of interneurones co-inhibited as determined by eqn. (3) against the percentage determined by eqn. (2), as A varies. It is clearly apparent that the distribution of inhibition determined by an interneuronal pool the connectivity of which is entirely random will not be as we initially expected. If this is the case, then we may expect the 'observed' *vs.* 'predicted' proportions of Fig. 4 to deviate from the continuous line. Indeed, using the values of B and N as determined above, gives a plot in Fig. 6 which is similar in form to that in Fig. 4*A*. Thus, the data of Fig. 4*A* are also compatible with an organization wherein individual presynaptic fibres randomly innervate the population of interneurones and these interneurones randomly innervate other interneurones of the population. By contrast, the much closer correspondence between observed and predicted frequencies which is present in Fig. 4*B*, is compatible with an organization in which the inhibitions evoked by group I and by non-muscular fibre types are evoked by completely separate groups of interneurones (marked P_3 in Fig. 5*C*).

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