J. Physiol. (1985), **368**, *pp.* 33–62
With 13 *text-figures*
Printed in Great Britain

THE DETECTION OF MONOSYNAPTIC CONNEXIONS FROM INSPIRATORY BULBOSPINAL NEURONES TO INSPIRATORY MOTONEURONES IN THE CAT

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(*Received 12 April 1985*)

SUMMARY

1. Simultaneous recordings were made of the discharges of inspiratory bulbospinal neurones and phrenic or external intercostal α-motoneurones in the anaesthetized cat. The connexions between these neurones were studied by the construction of cross-correlation histograms from their discharges.

2. Peaks observed in the cross-correlation histograms were divided into three groups on the basis of their time courses: narrow, medium-width and high-frequency oscillations (h.f.o.). Narrow peaks were defined as having half-widths less than 1·1 ms and medium-width peaks as having half-widths greater than this, while h.f.o. was characterized by periodic waves in the range 60–120 Hz.

3. H.f.o. peaks were interpreted as being derived from the well known periodic synchronization of medullary inspiratory neurones in this frequency range.

4. The time courses and latencies of the medium-width peaks could be quantitatively explained by a simple model representing excitation of the motoneurones by bulbospinal neurones whose discharges showed synchronization within ± 1 ms of the reference spike, together with temporal dispersion in bulbospinal axons having a distribution of conduction velocities given by the measurements of this study. Such an explanation was essential for some of the medium-width peaks, whose latencies were short compared to the conduction times to the spinal cord for their own axons, but for other medium-width peaks oligosynaptic excitation of the motoneurones from the identified bulbospinal neurones was another possible explanation.

5. The narrow peaks were of appropriate durations for monosynaptic connexions and were all at appropriate latencies (0·6–2·4 ms after the calculated arrival time of the bulbospinal impulse in the segment concerned).

6. It is concluded from the observations of narrow peaks that monosynaptic excitation exists between inspiratory bulbospinal neurones and both phrenic and external intercostal motoneurones. However, because of the existence of presynaptic synchronization, as shown by the presence of the medium-width peaks, such a conclusion is predicated upon being able to discriminate against such an effect. The model showed that this restriction applies just as much to the measurements of excitatory post-synaptic potentials (e.p.s.p.s) by spike-triggered averaging as it does

to cross-correlation measurements. We suggest that the discrimination against presynaptic synchronization here was possible only because the long conduction distance created temporal dispersion in the synchronized presynaptic impulses.

INTRODUCTION

Only a fraction of the total number of fibres synapsing on a spinal motoneurone may need to be active in order to depolarize its membrane from a resting state into its firing range (Kirkwood, Sears & Westgaard, 1984). The relatively slowly rising activation of phrenic and external intercostal motoneurones in a moderately deeply anaesthetized cat represents a situation where it may be possible to allocate particular fractions of this activation to one or another set of input fibres, each one firing at particular input rates with appropriate average sizes of unitary excitatory post-synaptic potentials (e.p.s.p.s). We believe this to be the case for these motoneurones because, provided the anaesthetic state is deep enough, the excitation mostly consists of the phasic inspiratory drive and it is derived directly or indirectly from the inspiratory bulbospinal neurones of the dorsal and ventral respiratory groups (d.r.g. and v.r.g.) in the medulla (Merrill, 1974; Kirkwood, Sears, Tuck & Westgaard, 1982*b*; cf. Kirkwood & Sears, 1973).

The question addressed in this and the following paper is, how much of the excitation of the motoneurones is derived monosynaptically from the bulbospinal neurones and, by exclusion, how much polysynaptically? For the phrenic motoneurones there is already plentiful evidence for the *existence* of a monosynaptic pathway from both cross-correlation measurements and spike-triggered averaging of synaptic noise (Cohen, Piercey, Gootman & Wolotsky, 1974; Hilaire & Monteau, 1976, 1979; Graham & Duffin, 1982; Feldman & Speck, 1983; Lipski, Kubin & Jodkowski, 1983; Fedorko, Merrill & Lipski, 1983; Cohen & Feldman, 1984). However, in these studies close attention was not given to the criteria for the identification of a monosynaptic connexion, particularly with regard to the possibility of synchronization of units within the medulla. No attempt has yet been made to assign a fraction of the total motoneurone depolarization to this input.

For external intercostal motoneurones the only support for a significant monosynaptic pathway comes from a cross-correlation study by Hilaire & Monteau (1976) together with indirect evidence from this laboratory (Kirkwood, Sears, Stagg & Westgaard, 1982*a*).

There are two principal methods available for this sort of study. One of them, spike-triggered averaging, may have some limitations due to restricted sampling. In the present study, we have made a more widespread investigation by the use of the second method, cross-correlation, but in a more critical fashion than hitherto, taking advantage of the increased understanding that has recently come about in the interpretation of cross-correlation histograms (Kirkwood, 1979; Kirkwood & Sears, 1982*a*, *b*; Fetz & Gustafsson, 1983; Gustafsson & McCrea, 1984) and taking particular care to allow for synchronization between units in the medulla. In this paper we show that there is indeed good evidence for a monosynaptic pathway and in the following paper (Davies, Kirkwood & Sears, 1985) we attempt an assessment of the over-all contribution of this pathway to the excitation of the motoneurones. We have

concentrated mostly on intercostal motoneurones, but data from phrenic motoneurones are included both for comparison with the intercostal ones and for comparison of this study with others in the literature.

Preliminary results have been published (Kirkwood, Sears & Stagg, 1974; Kirkwood & Sears, 1980; Kirkwood, Sears & Davies, 1983; Sears, Kirkwood & Davies, 1985).

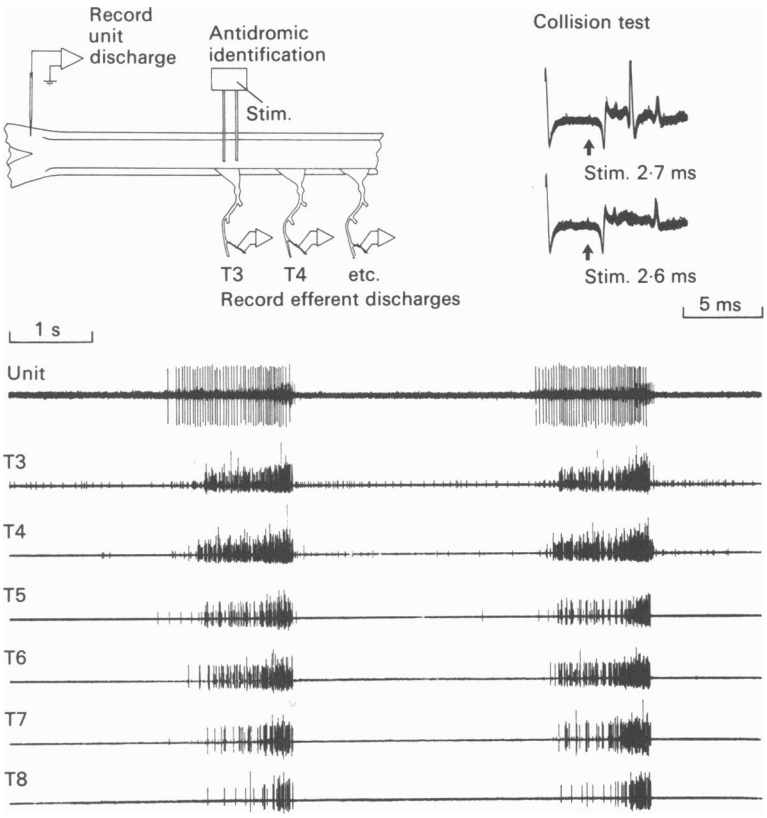

Fig. 1. Experimental arrangement and typical recordings. Although shown at T3, the most common site for the stimulating electrodes was T4–T5. A collision test is illustrated for the bulbospinal unit whose spontaneous activity is illustrated below (large single unit). A stimulus (Stim.) of twice threshold was used and is shown following a spontaneous spike at the two delays which straddled the critical delay. The small, longer-latency spike in the collision test records comes from the unit which appears as a late inspiratory discharge in the record below. This unit was also bulbospinal, as was confirmed by another collision test (critical delay 4·0 ms, not illustrated).

METHODS

Experiments were carried out on eighteen cats of either sex weighing 2·2–3·6 kg, anaesthetized with sodium pentobarbitone, paralysed with gallamine triethiodide and ventilated with air, O_2 or, most often, a 2:1 air/O_2 mixture. The anaesthesia procedures and care of the preparation were as described by Kirkwood *et al.* (1982*b*). Most of the animals here would have been described in that paper as being moderately deeply anaesthetized. When necessary (five occasions), systolic blood pressure was maintained above 100 mmHg by infusion of a noradrenaline–dextran solution.

In twelve of the animals, recordings of efferent inspiratory discharges were made from naturally

occurring filaments of the external intercostal nerves of up to six segments between T2 and T9 (most often T3–T8 or T3–T7) on one side of the animal. (For details and references see Kirkwood *et al.* 1982*a*, *b*.) Usually the most proximal (dorsal) filament of each segment was used, although when this was very thin or showed a weak discharge the two most proximal filaments were mounted together on one pair of electrodes. In order to promote inspiratory activity, particularly in the more caudal segments where it is usually weak in eupnoea, CO_2 was frequently added to the inspired gases so as to bring the end-tidal concentration into the range 5–7 %.

For eleven of the animals (including five of the twelve mentioned above) the efferent discharge was also recorded in the C5 phrenic root. The nerve was not desheathed (which should have helped to preserve the relationship between spike height and conduction velocity (see Davies *et al.* 1985)) but was carefully cleaned of most of the fat and connective tissue before being cut and mounted on electrodes.

The medulla was exposed by an occipital craniotomy with the head ventroflexed and the cerebellum gently retracted. Extracellular recordings, via glass micropipettes filled with 3 M-NaCl, were made from inspiratory bulbospinal neurones contralateral to the phrenic or intercostal nerves used for the recordings of efferent discharges (Fig. 1). In some animals 3 % agar in normal saline was used to mechanically stabilize the medulla. Units were identified as bulbospinal by stimulation of their axons via a bipolar electrode, consisting of a pair of insulated stainless-steel needles 3 mm apart with about 1 mm tip exposed, inserted into the ventrolateral funiculus of the upper thoracic spinal cord (usually T4–T5) on the side used for efferent recordings. For six animals where only phrenic discharges were recorded, the stimulating electrode was inserted in C3 spinal cord segment. Antidromic excitation of the neurones was identified by the usual criteria, including, in all cases, a collision test and confirmation that the critical delay in the collision test did not represent soma refractoriness by the use of double shocks to generate paired antidromic responses separated by an interval less than the minimum interval in the collision test.

The positions of the units relative to obex were calculated from the micromanipulator readings. Units were assigned to either the d.r.g. (in the region of the nucleus of the solitary tract, from 0·1 to 2·8 mm rostral and 1·9 to 3·2 mm lateral to obex) or the v.r.g (in the region of nucleus retroambigualis, from 1·0 caudal to 1·0 mm rostral and 2·8 to 4·3 mm lateral to obex). The co-ordinates of these two groups overlapped, largely due to inter-animal variation, although in one of the cats the two groups could be recorded at different depths on some of the same penetrations. In all cases the positions of the units along the penetrations, relative to the medullary surface, were appropriate to these two groups (see, e.g. Bianchi 1971; Merrill, 1974) although such absolute depth measurements were not always considered reliable. Other criteria were used to confirm the electrode position, such as the background noise of lung stretch afferents and baroreceptor afferents recorded when the electrode tip was in the solitary tract, or the location of the cuneate, gracile and trigeminal nuclei. The identification of none of the units was ambiguous, although some other inspiratory units in apparently intermediate regions between the d.r.g. and the v.r.g. were also recorded (cf. Kreuter, Richter, Camerer & Senekowitsch, 1977; Champagnat, Denavit-Saubié & Velluti, 1980; King & Knox, 1984). Post mortem, the distances from obex to each relevant spinal cord segment were measured along the cord dorsum and from the cord to the recording electrodes along the whole course of the phrenic or intercostal nerves.

The experimental procedure consisted of recording the unit discharges together with up to six channels of efferent motoneurone discharges on magnetic tape (band width, d.c. to 2·5 kHz) for off-line cross-correlation analysis by computer (DEC PDP-11/34). 10–96 min were recorded for each bulbospinal unit. Data were acquired for subsequent analysis via a purpose-built interface (Davies, Forster & Sears, 1983) which included window discriminators in each of eight channels so that the bulbospinal unit and the α (occasionally the γ) discharges from each nerve recording could be selected. The longest runs included totals of about 3 million spikes distributed between the seven channels. The times of the spikes (sampled at 32 μs) were stored in units of 10 μs as the times of the second crossing of the lower levels of the window discriminators. Single-unit spikes in the medulla were always of the negative–positive type. The negative phase was usually used for the window discriminator. If not, the spike times were adjusted to be equivalent to this. Due to the filtering in the tape recorder, the durations of the negative phases of the unit spikes and the monophasic efferent discharges were very similar, so the cross-correlation histograms between these times are the same as they would have been had the rising phases of the negative spikes in the medulla and the monophasic efferent spikes been used instead. Times in the collision tests were referred directly to the rising phases of the negative unit spikes.

Analysis consisted of constructing cross-correlation histograms between the times of occurrence of the bulbospinal unit spikes and the efferent spikes of each of the other channels. All the α spikes from each filament were generally taken together as one (multi-unit) population. The bin width for these histograms was usually 0·192 ms, although sometimes bins were later combined to give a wider bin width. The auto-correlation histograms were also calculated (at this bin width) for each unit discharge. In addition, cross-correlation histograms (usually with a bin width of 1 ms) were calculated between selected intercostal recordings (always including a pair of adjacent segments, usually the most rostral). Some analysis was performed during the experiment to help to determine the protocol, particularly with respect to levels of anaesthesia (see Results).

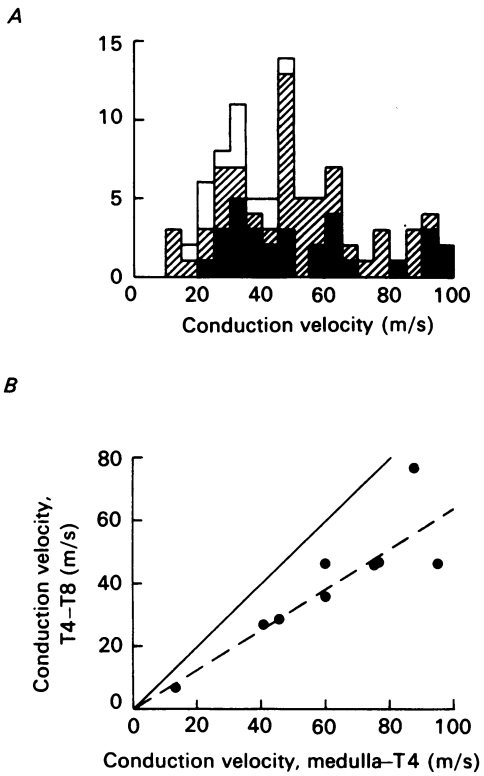

Fig. 2. *A*, distribution of the conduction velocities of all the bulbospinal units with axons identified at thoracic levels. Filled, d.r.g.; hatched, v.r.g.; open, v.r.g. (less accurate values from two experiments, due to the possible use of a stimulus less than twice threshold). *B*, demonstration of slowed conduction in nine bulbospinal axons in one experiment. The calculated conduction velocity between T4 and T8 is less than that between the medulla and T4. The lines have slopes of 1·0 and 0·638 (the mean ratio for the nine pairs of measurements).

RESULTS

Properties of the bulbospinal neurones

A total of eighty-seven inspiratory neurones with axons extending at least as far as the upper thorax were identified (thirty in the d.r.g., fifty-seven in the v.r.g.). Of these, sixty were used for cross-correlation measurements (nineteen in the d.r.g., forty-one in the v.r.g.). The remaining twenty-seven were used only for conduction velocity measurements. Conduction velocities were estimated from the collision test

as follows. A value of stimulus strength of exactly twice threshold was consistently used for the cord stimulus and the critical delay between the times of occurrence of a spike in the medulla and the cord stimulus was determined in the usual way. The refractory period (relative) was assumed to be 0·5 ms, giving the conduction time simply as 0·5 ms less than the critical delay. This procedure gave a direct estimate of *orthodromic* conduction time measured during the period when the cell was firing at physiological rates and using the same reference time (the rising phase of the spontaneous spikes) as was used in the cross-correlation analyses. The likely error (0·1–0·2 ms) is less than other measurements based on *antidromic* conduction times. These latter times show a great deal of variation and are not very closely linked to the orthodromic conduction times (Fuller & Schlag, 1976; Rapoport, Susswein, Uchino & Wilson, 1977; Lipski, 1981). Conduction velocities were not calculated for another nine neurones identified with a cord stimulus at C3 level.

Measured velocities for all the bulbospinal neurones projecting to the upper thoracic cord are shown in Fig. 2*A*. Some of the values from the v.r.g. (shown unshaded, thirteen cells) may be inaccurate because in two experiments some stimuli less than twice threshold were used. The conduction times in these experiments were therefore probably slightly over-estimated and thus the conduction velocities slightly underestimated. When these values are excluded the mean conduction velocities were 53·5 m/s for the d.r.g. and 50·1 m/s for the v.r.g., which are not significantly different. The conduction velocities were used to calculate appropriate latencies for the cross-correlation measurements, but for this some assessment of the degree of slowing of conduction along the course of the axons was also required. In one cat we therefore placed two pairs of stimulating electrodes, one at caudal T4 and one at caudal T8. Of the thirteen bulbospinal units identified from T4, nine were also identified (by the same criteria) from T8. At least 69 % of axons that extend to T4 therefore also extend to T8, but because the electrodes at T8 were fixed and a search for the axons at T8 was not made, it is quite possible that the remaining four axons also extended to the caudal thorax.

The conduction velocities between T4 and T8 (calculated from the difference between the critical periods of the two collision tests) were all less than those calculated for conduction between the medulla and T4. The relationship between the two velocities is illustrated for these nine cells in Fig. 2*B*. The reduction in conduction velocity is quite consistent, giving a mean value between T4 and T8 of 63·8 % of that measured more rostrally.

Another property of the bulbospinal units that we wish to use in order to estimate the effectiveness of their connexions (Davies *et al.* 1985) is their mean firing frequency at the time most of the motoneurones were recruited. The firing frequencies were all maximum or nearly maximum near the end of inspiration. Most of the units fired throughout inspiration, although a few fired only in about the last third. Some continued to fire in early expiration ('post-inspiration', Richter & Ballantyne, 1983). An approximate value for the firing frequency of each neurone near its maximum was calculated from its auto-correlation histogram (cf. Figs. 4–6; figs. 1 and 2, Davies *et al.* 1985) as the reciprocal of the interval corresponding to the first peak or the shoulder (e.g. fig. 2, Davies *et al.* 1985) if no sharp peak was present. These frequencies are appropriate to the latter part of inspiration when most of the

motoneurone recruitment has already occurred. Values ranged from 33 to 400 impulses/s, mean 99±61 (S.D.) impulses/s ($n = 68$).

Types of cross-correlation histogram peaks defined by their time courses

Many of the cross-correlation histograms calculated between the discharges of single bulbospinal neurones and intercostal or phrenic nerves showed either single or multiple peaks. These features could be divided into three types on the basis of their time courses. Each type, as defined below, sometimes appeared in isolation, although more often in combination. To avoid confusion, it should be noted that the definitions

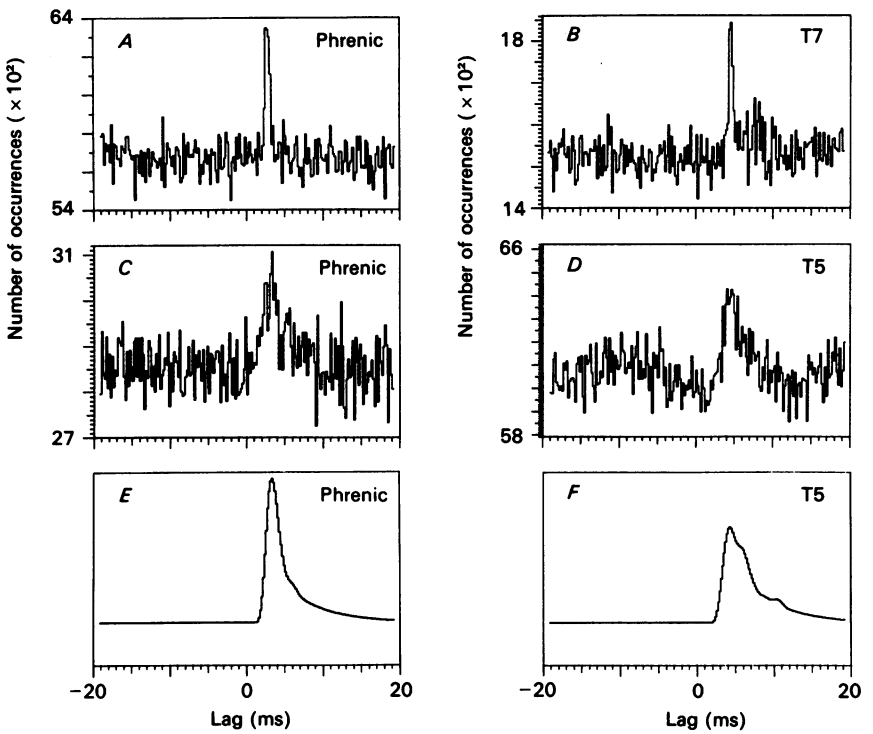

Fig. 3. *A–D*, typical examples of peaks in cross-correlation histograms constructed from the discharges of individual bulbospinal units (reference spikes) and motoneurone discharges. Each histogram comes from a different bulbospinal unit; *A* and *C*, phrenic discharges; *B* and *D*, intercostal discharges from the indicated segments. *A* and *B*, narrow peaks; *C* and *D*, medium-width peaks. See text for definitions of types of peaks. Conduction velocities and locations of units: *A*, 100 m/s (d.r.g.); *B*, 47·3 m/s (rostral v.r.g.); *C*, 28·0 m/s (d.r.g.); *D*, 72·2 m/s (caudal v.r.g.). Numbers of reference spikes: *A*, 52462; *B*, 55215; *C*, 40623; *D*, 98675. *E* and *F*, theoretical time courses for medium-width peaks for phrenic or T5 intercostal discharges. See Appendix or text for explanation.

below are independent of the definitions established for intercostal motoneurone synchronization, namely short-term synchronization (Sears & Stagg, 1976), broad-peak synchronization and h.f.o. (Kirkwood *et al.* 1982*a*, *b*).

(*i*) *Narrow peaks.* As justified later, peaks were classified as narrow if their half-widths were less than 1·1 ms. Typical examples for the phrenic and for intercostal

discharges are shown in Fig. 3*A* and *B*. These peaks all had positive lags of a few milliseconds and were taken as indications of monosynaptic connexions from the bulbospinal neurones to the motoneurones. A peak of this duration is appropriate to an increased probability of firing mostly during the rising phase of an underlying fast rising unitary e.p.s.p., whereas a polysynaptic connexion would involve some temporal dispersion in the various interneurones involved and lead to a more slowly rising e.p.s.p. and hence a longer duration peak (Kirkwood, 1979; Kirkwood & Sears, 1982*a*, *b*; Fetz & Gustafsson, 1983; Gustafsson & McCrea, 1984).

(*ii*) *Medium-width peaks.* Peaks were classified as medium-width if their half-widths were greater than 1·1 ms (typical half-widths were 2–5 ms). Two examples are shown in Fig. 3*C* and *D*. Two mechanisms may be hypothesized for the origins of these peaks: first, oligosynaptic connexions between the bulbospinal unit and the motoneurones, the width of the peak being dependent on the temporal dispersion of impulses in the various interneurones involved; secondly, presynaptic synchronization, i.e. synchronization of the bulbospinal unit's spikes with spikes of other cells that synapse on the motoneurones, the width of the peak being then dependent on the time course of the synchronization (in general aperiodic) and on the temporal dispersion of impulses in the axons of these other cells.

(*iii*) *High-frequency oscillations* (*h.f.o.*). This type of histogram was characterized by periodic waves with frequencies in the range 60–120 Hz. The largest amplitude wave always had a positive lag of a few milliseconds, i.e. compatible with an origin in a mono- or oligosynaptic connexion from medulla to motoneurones, as described for the phrenic (Cohen, 1979; Cohen & Feldman, 1984; cf. intercostal motoneurone synchronization, Kirkwood *et al.* 1982*a*, *b*). The immediate origin of the phenomenon almost certainly lies in the synchronization of inspiratory neurones in the medulla in this frequency range. Such synchronization prevents one from deriving useful information about the connexions of the selected bulbospinal neurone and in general we regarded h.f.o. as a contaminant, using on-line histograms in order to detect its presence. Attempts to suppress h.f.o. by increasing the depth of anaesthesia were made and were usually successful, but often at the cost of depressing intercostal efferent activity, especially in the more caudal segments. In order to maintain sufficient activity from all segments for the cross-correlation measurements, a compromise dose was usually used, resulting in the presence of a small amount of h.f.o. in many of the cross-correlation histograms. Examples of h.f.o. are included in Figs. 4, 6 and 7.

Evidence that presynaptic synchronization is involved in the generation of medium-width peaks

The most likely neurones that could be synchronized with a given bulbospinal neurone so as to generate a medium-width peak are other bulbospinal neurones, since several studies have shown them (or medullary inspiratory neurones in general) to be synchronized (Vachon & Duffin, 1978; Feldman, Sommer & Cohen, 1980; Graham & Duffin, 1982; Madden & Remmers, 1982; Feldman & Speck, 1983; Hilaire, Monteau & Bianchi, 1984). We therefore formulated a simple model (see Appendix and Fig. 11) to predict what shape of cross-correlation histogram peak might be expected from such synchronization. The most important assumption in the model is that

bulbospinal neurones over the whole range of conduction velocities are involved. Temporal dispersion (i.e. the distribution of conduction times from medulla to motoneurones) was calculated for each spinal segmental level from the distribution of conduction velocities shown in Fig. 2*A*. This distribution was convolved with an assumed time course for the synchronization in the medulla, and the result convolved with a time course for the raised probability of firing of a motoneurone evoked by individual presynaptic spikes (the primary correlation kernel) for each of these presumed descending axons. Allowance for a fixed delay at segmental level was also made.

The synchronization in the medulla could have various time courses. We chose the most likely time course to be a triangular increase in firing probability lasting ± 1 ms. For the primary correlation kernel of the motoneurones the most likely estimate was a peak with a half-width of 0·7 ms. The predicted shapes of the cross-correlation histograms for phrenic and T5 intercostal motoneurones for these conditions are shown in Fig. 3*E* and *F*. It should be noted that as the conduction distance down the cord increases (i.e. going from the phrenic to T5) both the latency and the half-width of the calculated peaks increase and there is close agreement between the predicted and the observed peaks (Fig. 3*C* and *D*).

Not surprisingly, other examples of medium-width peaks obtained experimentally did not fit so well with the predictions obtained with these particular time courses for medullary synchronization and primary correlation kernel. Often the shapes of the peaks were appropriate, but the latencies were different by 1–2 ms. This may readily be explained by assuming that the bulbospinal unit was synchronized with a slight positive or negative delay to the other bulbospinal neurones. This could arise if, for example, the particular bulbospinal unit itself excited a number of its fellows or conversely if it was excited by a number of them. Examples of synchronization between medullary neurones where the peaks are displaced from zero lag are not uncommon in the published descriptions (op. cit.). Similarly, a few examples of the medium-width peaks had appropriate latencies but longer half-widths, but these would be expected because the published descriptions of synchronization within the medulla include some examples with peaks wider than that chosen for the theoretical examples in Fig. 3*E* and *F*. (The durations being considered are still only a few milliseconds, as distinct from the tens of milliseconds involved in the broad-peak synchronization of Kirkwood *et al.* 1982*b*.)

Thus, via the model, presynaptic synchronization is at least a *sufficient* explanation for all of the medium-width peaks. It is also a *necessary* explanation for some of them. The critical measurement is latency, which allows the distinction to be made between presynaptic synchronization and oligosynaptic excitation. In this context we have included the situation where the unit used in the cross-correlation measurements excites other bulbospinal neurones (which then excite the motoneurones) under the heading of presynaptic synchronization rather than oligosynaptic excitation, because we wish to distinguish between medullary interneurones (e.g. bulbospinal neurones as interneurones) and spinal cord interneurones. The important difference between these two situations is that for presynaptic synchronization the latency is independent of the conduction velocity of the particular bulbospinal neurone, whereas for oligosynaptic transmission via spinal cord interneurones (or for a monosynaptic

connexion) it is not. Thus for the peaks in Fig. 3*E* and *F* the latency of the early foot of the peak is determined by the conduction time for the fastest units of the conduction velocity distribution (100 m/s) and is independent of the conduction velocity of the particular bulbospinal neurone.

For Fig. 3*C* and *E* these latencies are both about 1·5 ms. However, the unit involved in Fig. 3*C* had a slow conduction velocity (28 m/s) and the impulse in its axon would not reach segment C5 until 2·0 ms. In this case, then, the fact that the latency is even shorter than the conduction time is conclusive proof of presynaptic synchronization. Several of the examples of medium-width peaks demonstrated this property. In contrast, Fig. 3*D* was constructed from the activity of a fast-conducting unit (72 m/s) and the calculated conduction time to T5 was 1·9 ms. Thus the early part of the peak here (latency about 2·5 ms) is compatible with monosynaptic excitation and the later part with oligosynaptic excitation. Only the over-all shape of the peak and, in particular, the initial slowly rising foot makes one suspect presynaptic synchronization in this case. Readers should compare the theoretical examples, Fig. 3*F* here and fig. 7 of Kirkwood (1979), which each have a slowly rising foot, with the measurements of mono- plus polysynaptic excitation from spindle afferents in fig. 2 of Kirkwood *et al.* (1982*b*), which do not.

Thus, in summary, presynaptic synchronization is the simplest explanation for most of the medium-width peaks. For some of them the early latencies make this explanation absolutely necessary, but for others the participation of oligosynaptic excitation via spinal cord interneurones cannot be excluded.

Deductions made from periodic wave forms

Not all the histograms showing periodicities in the h.f.o. frequency range were classified as h.f.o. An example is Fig. 3*D*, which shows a medium-width peak. In fact, in many cases we believe medium-width peaks and h.f.o. were present together. These two are not easily separated: for the intercostals in particular, the predicted durations of the medium-width peaks are very close to half a cycle of h.f.o. However, if the bulbospinal unit fired periodically (i.e. regularly at a given frequency) then its own periodicity should appear in the cross-correlation histogram. If this periodicity has a different frequency from the h.f.o. then it should be possible to separate the two effects (Moore, Segundo, Perkel & Levitan, 1970). Unfortunately, the auto-correlation histograms from the bulbospinal neurone discharges were often either relatively flat for lags greater than the refractory period, corresponding to irregular or rate-modulated firing, or showed a periodicity very close to that of the h.f.o., so that only in a few cases could information be gained from the periodicities.

One of these cases is illustrated in Fig. 4; two unit/motoneurone histograms are shown, for T4 motoneurones (*A*) and for T5 motoneurones (*B*); a periodic wave (period about 13·5 ms) with a superimposed narrow peak is present in *A*, whereas *B* shows the periodic wave alone. The periodic wave does not reflect the unit's firing frequency, which was rather steady at around 150 impulses/s, as is shown by the clear periodicity (6·5 ms) in the auto-correlation histogram (Fig. 4*C*). However, the period of 13·5 ms does appear in the cross-correlation between the two groups of motoneurones (Fig. 4*D*). We can deduce that this periodicity is not just a feature of the motoneurone discharge because, if so, it would appear asymmetrically on the right of the

histograms in Fig. 4*A* and *B* (Moore *et al.* 1970), which is not the case. Rather, this periodicity must reflect the period of a common input to both the unit and the motoneurones, i.e. in this case it is h.f.o.

It is worth noting that the monosynaptic excitation represented by the narrow peak in Fig. 4*A* did not produce periodic peaks at 6·5 ms intervals as might perhaps be

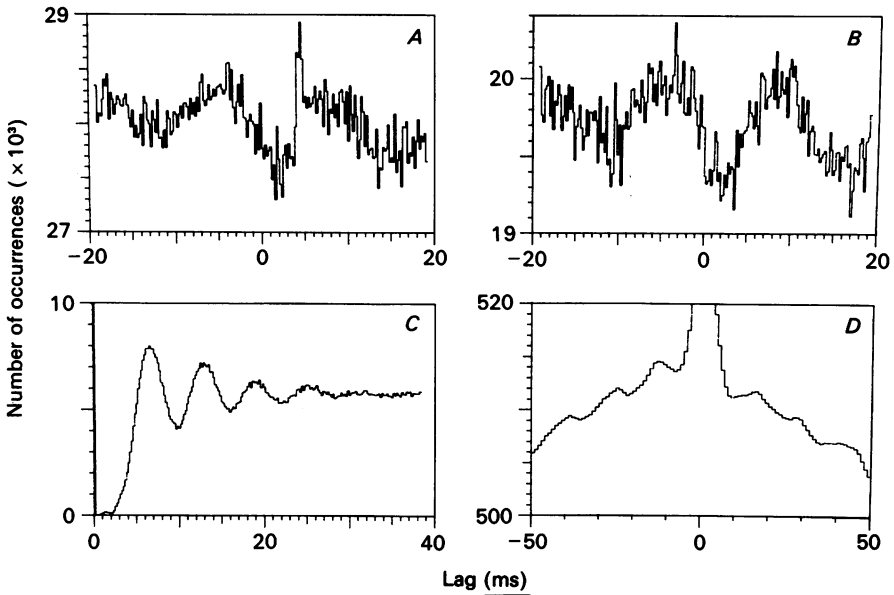

Fig. 4. Identification of h.f.o. *A* and *B*, cross-correlation histograms from the discharges of a bulbospinal unit in the caudal v.r.g. (conduction velocity 48·9 m/s) and intercostal discharges at T4 (*A*) or T5 (*B*). Histograms scaled in proportion to the base-line counts. The single bin at −3·7 ms in *B* is not significantly raised above its neighbours (*m* taken as 19950). See text for description of estimation of *m*. In contrast, all four bins of the narrow peak in *A* are significantly raised (*m* taken as 27950). *C*, auto-correlation histogram for the bulbospinal unit. *D*, cross-correlation histogram between the motoneurone discharges of T4 (reference spikes) and T5, from the same data as in *A* and *B* (histogram smoothed and truncated to show the small amplitude, but clear h.f.o. wave form). Numbers of reference spikes: *A–C*, 241997; *D*, 1463967.

expected. This is because the narrow peak has a rather small area. To a first approximation, any secondary peaks would have a similar area, but would be spread over a time corresponding to the duration of the first peak in the auto-correlation histogram, i.e. about 7 ms. Such a secondary peak would be within the noise on Fig. 4*A*. In fact periodic peaks which could be associated with narrow peaks were never seen, presumably for the above reason, although some of the h.f.o. wave forms often seen along with narrow peaks could well have included periodic components arising from the monosynaptic connexion but not detectable because the unit's own period was very close to that of the h.f.o.

Generally, the medium-width peaks (e.g. Fig. 3*C* and *D*) have a greater area than the narrow ones and any periodic components related to them should not be so much

attenuated. Some of the unit/motoneurone histograms with medium-width peaks might therefore be expected to show the converse of Fig. 4, i.e. a periodicity corresponding to the unit's firing frequency and not to h.f.o. However, none of the units giving medium-width peaks had periodic auto-correlation histograms with periods clearly different from h.f.o. Periodicities that were observed cannot therefore

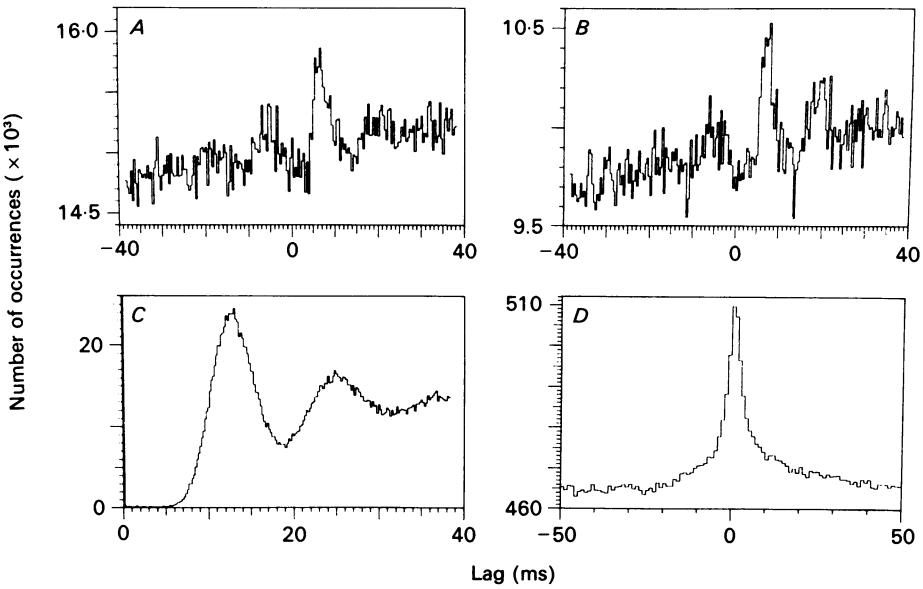

Fig. 5. Identification of a periodic effect which is probably not h.f.o. *A* and *B*, cross-correlation histograms for the discharges of a bulbospinal unit in the d.r.g. (conduction velocity 31·3 m/s) and intercostal discharges at T4 (*A*) or T5 (*B*). Histograms scaled in proportion to base-line counts. Note longer time scale and wider bin width (0·384 ms) than usual. *C*, auto-correlation histogram of the bulbospinal unit. *D*, composite cross-correlation histogram representing the sum of cross-correlation histograms between the intercostal motoneurone discharges of T3 and T4, T4 and T5, T5 and T6 (the first named of each providing the reference spikes). Note that despite the high sensitivity (large numbers of counts per bin) there is no sign of h.f.o. Numbers of reference spikes: *A–C*, 97261; *D*, 1485574.

be *conclusively* separated from h.f.o., although in at least two cases where periodicities were observed, no signs of h.f.o. were seen in the motoneurone synchronization. One of these cases is illustrated in Fig. 5; in both *A* (T4) and *B* (T5) the primary (medium-width) peak at a latency of about 4·5 ms is flanked on both sides by secondary peaks, giving a period of 12·5 ms, exactly the same as in the auto-correlation histogram for the unit (Fig. 5*C*). No such periodicity appeared in the cross-correlation histograms involving the motoneurones of adjacent segments (Fig. 5*D*).

In this example the symmetrical pattern of the secondary peaks suggests that medium-width peaks do not have an origin in aperiodic common inputs such as are postulated in the model, and which excite the periodic bulbospinal unit and the aperiodic motoneurone population, because that mechanism would give periodic peaks only to the left of the primary peak (Moore *et al.* 1970). Instead, the most likely

candidate (assuming the absence of h.f.o.) is oligosynaptic excitation of motoneurones from the bulbospinal neurone via spinal cord interneurones. Like the example of Fig. 3*D* the latencies are appropriate for this, despite the relatively low conduction velocity (31 m/s). For the example of Fig. 3*D* there was also no sign of h.f.o. in the motoneurone synchronization measurements.

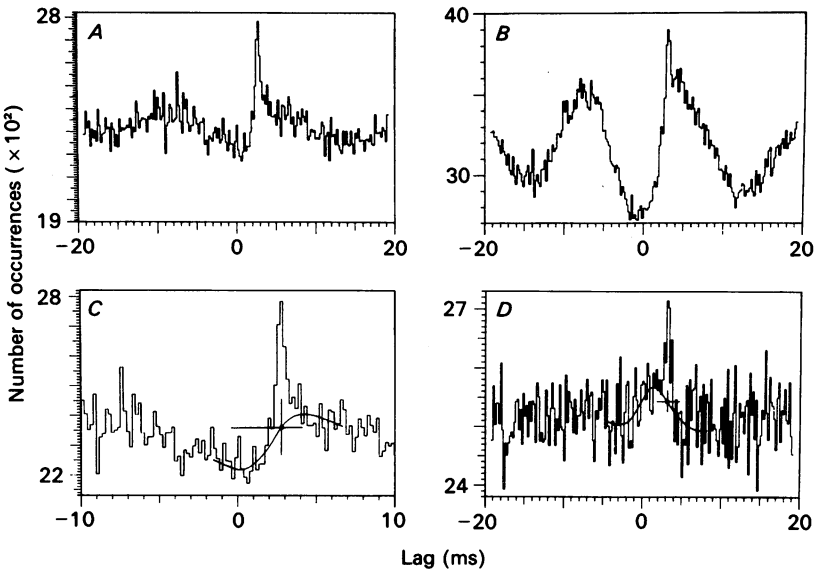

Fig. 6. Separation of narrow peaks from h.f.o. or medium-width peaks. *A* and *B*, cross-correlation histograms for the discharges of a bulbospinal unit in the d.r.g. (conduction velocity not measured) and the phrenic nerve; two different epochs analysed for the same unit. Histograms scaled in proportion to the base-line counts (2360 in *A* and 3350 in *B*). Note the narrow peak has a constant amplitude while the h.f.o. amplitude varies. *C*, the same histogram as *A* but on expanded scales to show the procedure for estimating the base-line counts, *m*, appropriate for the narrow peak (see text). *D*, an example with a different time relationship between narrow and medium-width peaks in a histogram for the discharges of another unit in the d.r.g. (conduction velocity 44·2 m/s) and the phrenic nerve. This peak is only just significant but is clear because of the several bins involved (cf. Fig. 7*C* and *D*). Base line fitted by eye. Numbers of reference spikes: *A* and *C*, 96629; *B*, 187059; *D*, 33972.

Measurements on narrow peaks in the presence of h.f.o. or medium-width peaks; estimation of significance and amplitude

In order to assess the monosynaptic connectivity between the bulbospinal neurones and the motoneurones, measurements on the narrow peaks must be made independently of the other components in the histograms so that the statistical significance of the narrow peaks can be assessed and their amplitudes and half-widths measured. An empirical approach was taken, which is illustrated in Fig. 6; cross-correlation histograms between the activities of a single bulbospinal unit and the phrenic nerve are shown in *A* and *B*, representing two separate experimental runs involving the same unit; these histograms are scaled in proportion to the base-line counts. The histograms demonstrate two components, a narrow peak with the same amplitude

in *A* and *B*, and h.f.o. which varied between the runs, presumably as the level of anaesthesia became lighter. The variation in level of anaesthesia is useful here in showing that the h.f.o. could vary independently of a narrow peak.

Fig. 6*C* shows the same histogram as in Fig. 6*A*, but on different scales in order to show the procedure for fitting a base line, from which the amplitudes of narrow peaks (and hence their significance and their half-widths) were measured. The base-line count (m) was given by the count at the intersection of a smooth curve drawn by eye to fit the underlying h.f.o. or medium-width peak and the vertical line through the maximum of the presumed narrow peak. Narrow peaks were only accepted as genuine if they were significant according to the criterion used by Sears & Stagg (1976), i.e. if they exceeded the base-line count by $3{\cdot}29\sqrt{m}$.

The test was used by Sears & Stagg (1976) for histograms with a flat base line on the reasonable assumption that the null condition (no peak) was represented by a Poisson distribution of counts per bin, where one bin in 2000 will exceed the chosen confidence limit by chance. The test was modified by Kirkwood *et al.* (1982*a*) to take account of the respiratory non-stationarity, which appears as a broad curvature in the base line of some of the cross-correlation histograms between motoneurones. Here it is further modified to take into account the h.f.o. or medium-width peaks. The test assumes that the underlying h.f.o. or medium-width peak does not introduce any more variability into the bin counts than is expected from the Poisson distribution for the observed increase in mean counts during such a peak. There is no theoretical justification for this, but the peaks assessed as significant by this test corresponded well with those that were significant by eye (Fig. 7*C* and *D*; fig. 2 of Davies *et al.* 1985) (cf. Kirkwood & Sears, 1982*b*).

Generally, the separation of narrow peaks by such methods presented little or no difficulty (see also Fig. 6*D* where the narrow peak is located on the falling phase of a medium-width peak), although the full time course of the narrow peaks often remained slightly uncertain. For instance, it is not clear whether the narrow peak in Fig. 6*C* has a 'tail' following the main narrow part, like the similar peaks from muscle spindle afferents (Kirkwood & Sears, 1982*b*), or whether there is a medium-width peak present as well as the narrow peak and the h.f.o. (see also Fig. 4*A*).

Occasionally this problem was more severe, particularly when the presumed narrow peak was superimposed on the leading edge of a presumed medium-width peak, as shown in Fig. 7*A*. This is the most dubious example of a histogram accepted as showing a narrow peak. A medium-width peak of the same duration as predicted from the model was assumed (drawn in) in order to calculate the base line. It is clear that this procedure of drawing in the medium-width peak is rather arbitrary in this case, even though the *presence* of the medium-width peak is very likely because of the troughs preceding and following the main peak. The presence of a narrow peak is indicated both by eye and because the over-all half-width (base-line count = 4490) was still less than 1·1 ms (see next section).

In contrast, in the examples of Fig. 3*C* and *D* no narrow peaks were identified, despite the fact that there might appear to be some narrow components. Reasons for this are first that the peaks here could be fitted over-all by the predictions from the model for presynaptic synchronization and secondly that the over-all half-widths were greater than 1·1 ms. However, in the assessment of connectivity which follows

(Davies *et al.* 1985), these examples could not be safely classified as *not* showing narrow peaks, and were instead classified as 'not tested' for narrow peaks. In all, only nine histograms (five intercostal, four phrenic) fell into this category. Histograms such as those in Fig. 7*B*, where the medium-width peaks or h.f.o. had a fairly smooth contour, were counted as not showing a narrow peak.

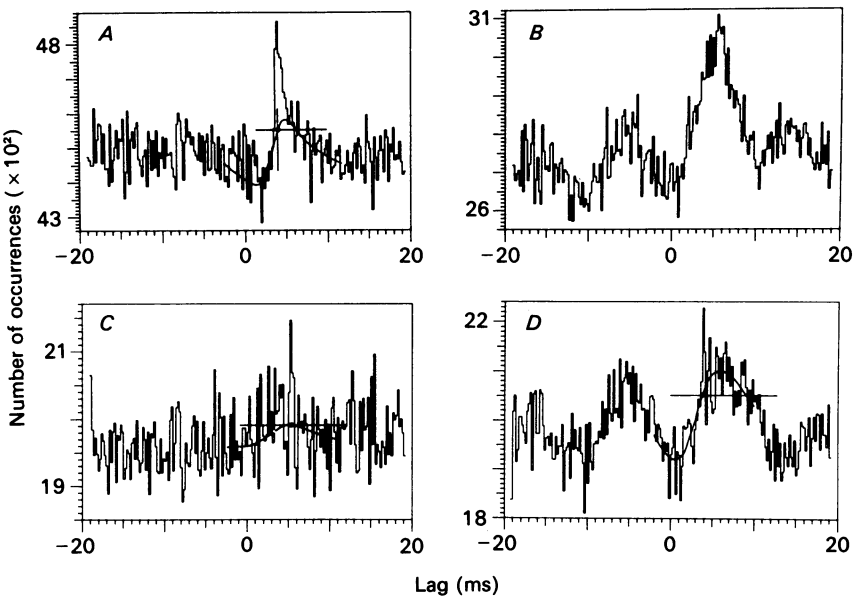

Fig. 7. Further examples of classification of peaks. Cross-correlation histograms for the discharges of four different bulbospinal units and intercostal nerves. *A*, slightly uncertain separation of narrow and medium-width peaks. Base line fitted by eye (see text). *B*, example of a histogram with a medium-width peak which was classified as the absence of a narrow peak. *C* and *D*, examples of narrow peaks which are only just significant (and only just detectable). Base lines fitted by eye. Conduction velocities and locations of bulbospinal units, intercostal segment and numbers of reference spikes: *A*, 65·0 m/s, caudal v.r.g., T6, 106910; *B*, 35·8 m/s, caudal v.r.g., T3, 115698; *C*, 24·4 m/s, rostral v.r.g., T3, 15816; *D*, 47·3 m/s, rostral v.r.g., T4, 35776.

The remaining two examples, Fig. 7*C* and *D*, are included to illustrate narrow peaks at the limit of significance, first where the base line is relatively flat (*C*) and secondly where there was moderately strong h.f.o. (*D*). In each of these examples one bin just exceeds the confidence limit with respect to the base line (drawn in), but that bin is also supported by one or two neighbouring bins with high counts. All cases where *any* bin exceeded the confidence limit (given the above provisos concerning medium-width peaks) were counted as narrow peaks, as long as their latencies were greater than zero (the time of the medullary spike) but less than the calculated conduction time to the appropriate segment of the spinal cord plus 3·5 ms. These peaks constitute the population described in the following sections and the following paper (Davies *et al.* 1985). There were also a few bins outside this latency range which exceeded the confidence limits (some in the positive direction and some in the negative one) but

no more than would be expected by chance. This latter group never included pairs of adjacent bins and they were ignored (cf. Kirkwood & Sears, 1982*b*).

The amplitudes of the narrow peaks were measured by the ratio k, the maximum count in the peak divided by m, in the same way that motoneurone synchronization and the strength of muscle spindle connexions have been assessed (Sears & Stagg, 1976; Kirkwood & Sears, 1982*b*; Kirkwood *et al.* 1982*a*). Values of k here ranged from 1·309 to 1·022. The lower limit must be presumed to be arbitrary; only a few histograms had sufficient counts/bin (> 22000) to be able to detect a peak with a value of k as low as 1·022. Mean values of k for the peaks observed were 1·114 for the intercostals ($n = 50$) and 1·113 for the phrenic ($n = 13$).

Durations of the narrow peaks

Durations of the narrow peaks were estimated as half-widths, i.e. the duration at half-amplitude. Alternatives such as the duration at the base were quite impractical in the presence of the other components described above. Half-widths were assessed to the nearest multiple of 0·096 ms (half a bin width) and account was taken of sloping base lines. For noisy histograms, interpolation was made by eye (e.g. Fig. 6*D*, half-width taken as 4 bin widths, 0·77 ms). In a few cases when the base line was very steep (e.g. Fig. 6*B*) the measurement of half-width was not considered very reliable. The same was true of several peaks which only just exceeded the confidence limits. These two categories were kept separate from the remainder.

In order to choose an upper limit of duration in the definition of a narrow peak we used the model described in the Appendix, because the principal effect we wished to discriminate against was presynaptic synchronization. From this model, the narrowest possible peak is that represented by the histogram of conduction times alone. This had a half-width of about 1·15 ms (6 bin widths) for the phrenic. We have taken this as representing the narrowest possible medium-width peak and the definition of a narrow peak is thus one with a half-width less than 1·1 ms. Interestingly, when histograms of the measured half-widths were plotted (Fig. 8) it was found that 1·1 ms neatly encompassed the main peaks both for the phrenic and for the intercostals. In these histograms the unshaded bins in the histograms relate to those peaks like Fig. 3*C* and *D* with half-widths below 2 ms but which were readily fitted by the model for presynaptic synchronization. All other medium-width peaks had half-widths greater than 2 ms.

Two points are worth noting. First, for the phrenic the dividing line of 1·1 ms is clearly slightly arbitrary, but the unimodal nature of the histogram, together with its similarity to that for the intercostals, suggests that not many peaks were wrongly classified. Secondly, the lack of medium-width peaks with half-widths below 1·6 ms for the intercostals when several are present for the phrenic is consistent with the interpretation that most of the medium-width peaks arise from presynaptic synchronization, the longer duration for the intercostals then resulting from the greater temporal dispersion in the conduction to the thoracic as opposed to cervical spinal cord.

Despite these uncertainties for the borderline cases, the main result of a consistent, unimodal distribution of half-widths (means: 0·63 ms, $n = 31$, for the intercostals; 0·68 ms, $n = 13$, for the phrenic) gave us confidence that this measure alone is a

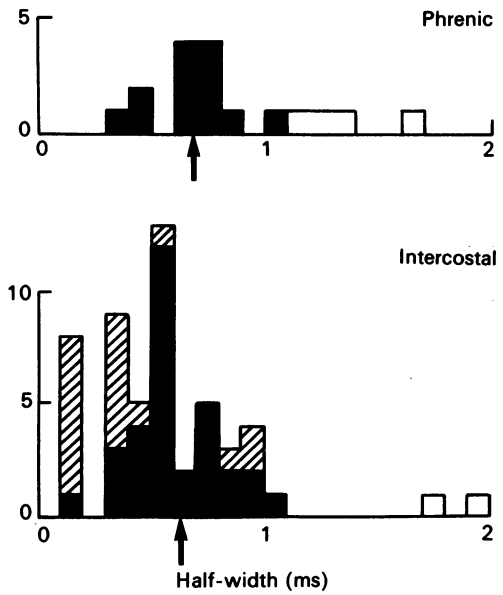

Fig. 8. Distribution of half-widths for all narrow peaks (filled or hatched columns) and for medium-width peaks with half-widths below 2 ms (open columns). Hatched columns represent narrow peaks where the half-width measurement was inaccurate because of a poor signal-to-noise ratio or because of underlying medium-width peaks or h.f.o. Arrows indicate the means of the half-widths represented by the filled columns.

reasonable indicator of a monosynaptic connexion, as is further justified by the latency measurements which follow.

Latencies of narrow peaks

Narrow peaks usually consisted of a few bins with high counts, even if only one of these had a count exceeding $m+3{\cdot}29\sqrt{m}$. The latency was taken as the start of the bin which, by eye, was the first of these bins with high counts. A basic requirement for monosynaptic connectivity is that the latency should be related to the axonal conduction velocity of the bulbospinal unit. This requirement was satisfied by the narrow peaks at all segmental levels, as shown in Fig. 9. At each level the latency decreases with conduction velocity and for a given conduction velocity the latency increases with distance from the medulla.

In order to confirm that the latencies were appropriate for monosynaptic connexions the 'transmission delay' was calculated. This is the latency measured from the time of arrival of the bulbospinal unit's impulse at the spinal segment, as calculated from the collision test (see earlier section). This delay includes slowed conduction in collateral branches (Merrill, 1974; cf. Munson & Sypert, 1979), synaptic delay and conduction time along the motoneurone axons. The conduction distance from spinal cord to the phrenic recording site was about the same as that for the intercostals (about 30 mm).

Values of transmission delay for the phrenic are shown in a histogram (Fig. 10*C*) and for the intercostals as a scatter diagram with segmental level along the abscissa

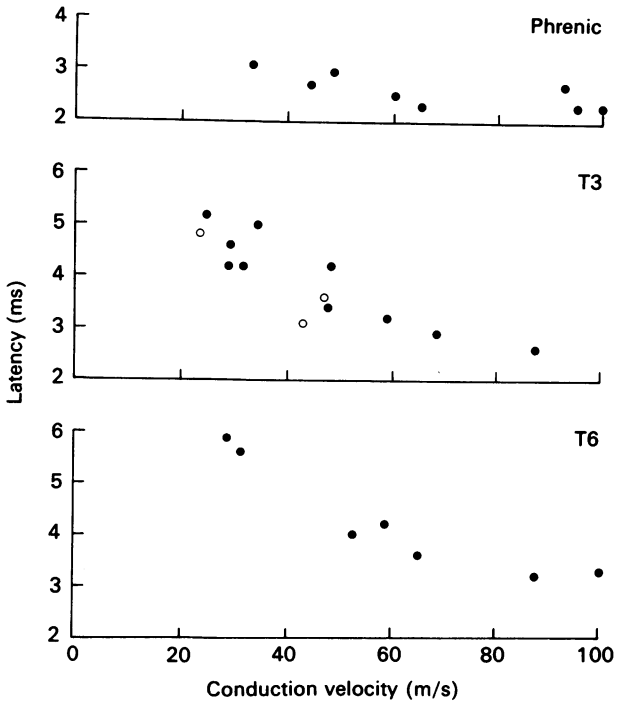

Fig. 9. Latencies of all narrow peaks seen for phrenic or T3 and T6 intercostal discharges, plotted against the conduction velocities of the bulbospinal axons as estimated from the collision tests. ○, values from the two experiments where the conduction velocities may have been underestimated.

(Fig. 10*A*). These values were calculated from a single value for each conduction velocity and demonstrate a clear tendency for the more caudal segments to show a longer delay. The regression line shown has a slope of 0·095 ms/segment, which is significantly different from zero ($P < 0{\cdot}01$). This tendency was expected because of the axonal slowing shown in Fig. 2*B*. Corrected transmission delays were therefore calculated by assuming two conduction velocities, one as measured by the collision test, for the phrenic and the intercostal segments above the level of the stimulating electrodes, and another, of value 0·638 times the first value, for more caudal levels.

The corrected times are shown in Fig. 10*B*, which has a regression line with a slope not significantly different from zero ($P > 0{\cdot}05$). These times are replotted as a histogram (Fig. 10*D*), which is very similar to that for the phrenic (Fig. 10*C*). The mean delay was $1{\cdot}54 \pm 0{\cdot}29$ (S.D.) ms ($n = 8$) for the phrenic and $1{\cdot}47 \pm 0{\cdot}32$ ms ($n = 37$) for the intercostals. Not only are these values internally consistent, being similar for both phrenic and intercostals, but they are also of an expected magnitude: to a first approximation 0·5 ms can be assigned to each of the three components of the delay, slowed conduction in bulbospinal axon collaterals, synaptic delay and conduction in motoneurone axons, giving a total of 1·5 ms. Moreover the range of delays is also remarkably close to the equivalent delays for muscle spindle afferents (Kirkwood & Sears, 1982*b*) which were measured from the time of cord entry. That

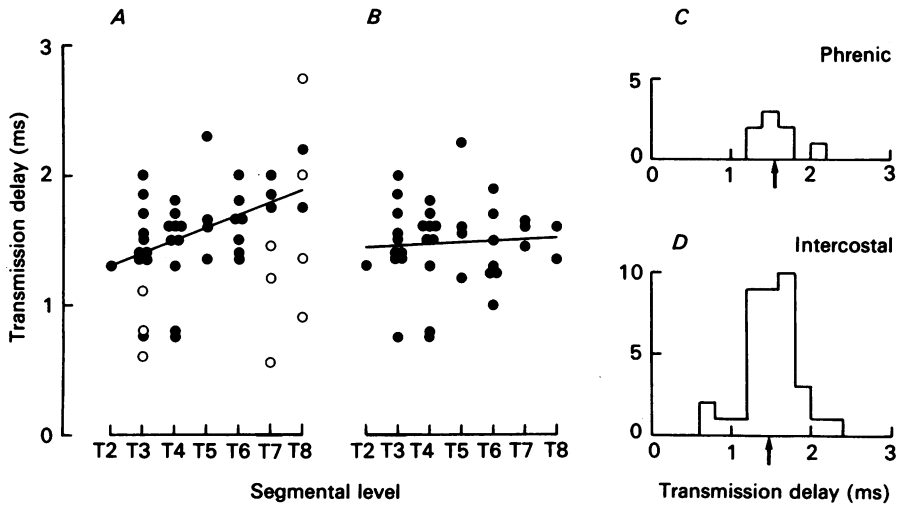

Fig. 10. Transmission delays (the differences between the latencies for the narrow peaks and the calculated arrival times of the bulbospinal impulses in the spinal cord segments concerned). *A*, for intercostal discharges; arrival times of bulbospinal impulses calculated from the single values of conduction velocity derived from the collision test. ○, values from the two experiments where conduction velocities may have been underestimated. *B*, corrected delays for the same peaks as in *A* (● only) calculated on the assumption of two conduction velocities for each bulbospinal axon: the same values as in *A* for the cord between the medulla and the segment where the axons were stimulated, 0·638 times those values more caudally. The lines in *A* and *B* are regression lines for the filled points. The slope is significantly different from zero in *A* but not in *B*. *C*, for phrenic discharges. *D*, values from *B* replotted as a histogram. Arrows in *C* and *D* show the means.

range was 1·1–1·9 ms, but dependent on the conduction velocity of the afferent. No dependence of the transmission delay on axonal conduction velocity was detected here.

DISCUSSION

The critical step in the analyses described here was the isolation of the narrow peaks. Both latency and half-width measurements independently identify them as an indication of a monosynaptic connexion. These peaks are present with very similar distributions of properties (half-widths, amplitudes and transmission delays) for both phrenic and intercostal motoneurones and one must conclude that a monosynaptic connexion is present for both groups. The physiological strength of these connexions is dealt with in the following paper (Davies *et al.* 1985).

Our conclusions are based partly on theoretical expectations for a cross-correlation histogram from a monosynaptic excitatory connexion and partly on comparisons with previous measurements on the well recognized monosynaptic link between muscle spindle afferents and motoneurones (Kirkwood & Sears, 1982*b*). They are also based on consideration of the *population* of narrow peaks. For an individual narrow peak one can *never* rule out completely the possibility that it has arisen from a connexion to the motoneurones from a few other neurones tightly synchronized with the bulbospinal neurone concerned. This possibility exists in any cross-correlation or

spike-triggered averaging study when the reference spike is derived from a central neurone. However, the whole set of properties illustrated in Figs. 8–10 makes it most unlikely that the majority of the narrow peaks derived from anything other than monosynaptic connexions from the identified neurones.

The mean half-width of the narrow peaks was 0·63 or 0·68 ms, which corresponds to an underlying average e.p.s.p. of about 1 ms rise time (cf. Fig. 13). This is fairly long for the rise time of a unitary e.p.s.p. but it should be remembered that the measurements here were made on multi-unit populations and that temporal dispersion both in presynaptic branches to the different motoneurones and in the motoneurone axons must be allowed for (Davies *et al.* 1985). In theory a half-width of 1·1 ms could allow for another synaptic delay and thus for the narrow peaks to include disynaptic as well as monosynaptic effects. However, we think this to be unlikely, first because the distribution of half-widths would then be expected to show a considerable tail extending beyond 1·1 ms, as a result of temporal dispersion in the interneurones involved in the majority of the disynaptic connexions. Secondly, the narrow peaks here correspond to the narrowest peaks in the earlier measurements involving muscle spindle afferents (Kirkwood & Sears, 1982*b*), which were believed to arise in the more deeply anaesthetized animals where polysynaptic effects were least evident (Kirkwood *et al.* 1982*b*).

Presynaptic synchronization and comparisons with the literature

It was essential for our conclusions that the effects of presynaptic synchronization (the medium-width peaks) could be detected and therefore separated from the peaks we wished to measure. Two measurements made this discrimination possible, the latency and the duration (half-width) of the peaks, and our success in using them depended on the long conduction distance between the medulla and the motoneurones. Without that long distance, the latency measurements would have been dominated by the variable segmental transmission delay and hence uselessly inaccurate. Without that long distance, the temporal dispersion in the other axons involved in presynaptic synchronization would have been absent and the medium-width peaks would have had durations which overlapped with those of the narrow peaks.

The knowledge that presynaptic synchronization can show up so readily in cross-correlation histograms throws doubt on the usefulness of this method for making precise measurements of the projections of *any* central neurones when such a range of conduction times is *not* involved. An exception to this could be made if one were totally certain that presynaptic synchronization did not exist (usually very difficult to determine) or if the durations of the cross-correlation peaks were *very* short (say, less than 0·5 ms). As we show below, this restriction applies just as much to studies using spike-triggered averaging as to those using cross-correlation. The difficulty arises not so much in the use of the measurements to prove the *existence* of a connexion but in the temptation to use the spike-triggered averaging method to establish average amplitudes of p.s.p.s or projection frequencies, such as can be done with more confidence for peripheral afferents, where presynaptic synchronization can be more easily eliminated (cf. Kirkwood & Sears, 1982*a*; Hamm, Reinking, Roscoe & Stuart, 1985).

Thus comparison of the results here with previous studies of connexions between

inspiratory neurones in the medulla and motoneurones is difficult because no previous author has attempted to separate the effects of presynaptic synchronization from direct effects (except for h.f.o.). The first of the two vital measurements, latency, has been considered along with measurements of the conduction velocities of individual bulbospinal neurones only in two recent spike-triggered averaging studies (Lipski *et al.* 1983; Fedorko *et al.* 1983). However, in both of these studies relatively inaccurate methods were used, which involved antidromic instead of orthodromic conduction times and a stimulation site away from the stem axon but not near the sites of recording of the e.p.s.p.s. Rather inconsistent values of latency for the e.p.s.p.s were therefore observed. The consistency and appropriateness of the latencies measured in the present study therefore themselves constitute a new result.

The second vital measurement, duration of the cross-correlation peak, has been measured in most of the previous studies. However, in some of them (Cohen *et al.* 1974; Hilaire & Monteau, 1976, 1979; Lipski *et al.* 1983; Fedorko *et al.* 1983) the cross-correlation has been performed by averaging the whole phrenic (or intercostal) nerve discharge, which may well have distorted the shape of the correlogram peak, particularly if the individual spikes in the recordings were slightly diphasic (cf. Kirkwood, 1979, fig. 8). Moreover, many of the illustrated peaks for the phrenic (e.g. Cohen *et al.* 1974, fig. 2; Lipski *et al.* 1983, fig. 7, C5) could be fitted quite well by the model presented here for presynaptic synchronization. The only examples for the intercostals (Hilaire & Monteau, 1976) have peaks which are far too wide for a monosynaptic connexion by our criterion and are best fitted by h.f.o. In other cross-correlation studies which use the same technique as here, where spikes are taken as point events for cross-correlation histograms, the duration of some of the illustrated peaks (Graham & Duffin, 1982; Cohen & Feldman, 1984) or of the only one (Feldman & Speck, 1983) are also too long to fit our criterion for a monosynaptic connexion.

Thus from previously published cross-correlation studies the evidence for a monosynaptic connexion is slender and we believe the present work is the first single correlation study to provide proper evidence. Moreover, because we have demonstrated that the effects of presynaptic synchronization can appear in the cross-correlation histograms and indeed can fit many of the published records, none of these previous cross-correlation studies can be relied upon for any quantitative estimates, such as projection frequency, which we attempt to make from our own measurements in the following paper (Davies *et al.* 1985). For the inspiratory intercostal motoneurones the data presented here are the only reliable measurements of a monosynaptic connexion from the medulla.

Implications of presynaptic synchronization for spike-triggered averaging studies

Fedorko *et al.* (1983) also did not trust previously published accounts of cross-correlations, mainly because their own results with spike-triggered averaging of synaptic noise differed from the cross-correlation results. We will return to this issue in the following paper (Davies *et al.* 1985), but it is important to realize that spike-triggered average e.p.s.p.s are as susceptible to distortion from presynaptic synchronization as peaks in cross-correlation histograms. In the Appendix we compare the effects of the same degree of presynaptic synchronization on a spike-triggered average of synaptic noise and on a cross-correlation histogram. In the

example shown (Fig. 13), because the component e.p.s.p.s have a greater duration than the component primary correlation kernels, the average depolarization due to presynaptic synchronization has a greater amplitude relative to the monosynaptic e.p.s.p. than the amplitude of the medium-width peak relative to the primary correlation kernel (cf. Kirkwood, 1979, fig. 7). Moreover the increase in half-width between the component e.p.s.p. and the average depolarization due to presynaptic synchronization is negligible compared to the great increase in half-width between the primary correlation kernel and the medium-width peaks in the cross-correlation histograms. Thus, by eye, the effects of presynaptic synchronization are easier to detect in the cross-correlation histograms than in spike-triggered average e.p.s.p.s.

The signal-to-noise ratio often appears greater for spike-triggered average e.p.s.p.s than for cross-correlation histograms. Certainly this is true if the same number of trigger spikes is employed in each of the two types of study. However, in terms of discriminating against the effects of presynaptic synchronization at least some of this increased signal-to-noise ratio is illusory because it is not the amplitude but the rise time of the e.p.s.p. that is critical for making this discrimination. The only way to be certain of the accuracy of measuring rise time is to differentiate the e.p.s.p. to see how much of the rising phase stands out above base-line noise. This differentiation process is a feature of the natural relationship between an e.p.s.p. in a cell and the raised probability of firing (Kirkwood, 1979; Gustafsson & McCrea, 1984) and so the two methods are, in the end, not so very different.

Further, because the over-all shapes of the average depolarization due to presynaptic synchronization and the monosynaptic e.p.s.p.s are so similar (Fig. 13), even if a sharply rising phase which one ascribes to a monosynaptic connexion is distinguishable (e.g. Lipski *et al.* 1983, fig. 7), uncertainty remains about how much of the rest of the e.p.s.p. may be derived from presynaptic synchronization. Again the two methods, spike-triggered averaging and cross-correlation, seem similar. This uncertainty in e.p.s.p. amplitude with spike-triggered averaging is matched by the uncertain relationship between amplitude of a cross-correlation peak and the amplitude of the underlying e.p.s.p. (Fetz & Gustafsson, 1983; Gustafsson & McCrea, 1984), which results from the dependence of the cross-correlation peak on the time differential of the e.p.s.p.

Effects on motoneurone synchronization

In addition to its significance for the detection of monosynaptic connexions, the occurrence of presynaptic synchronization could have one other consequence. Kirkwood *et al.* (1982*b*) showed that, under conditions of light anaesthesia, intercostal motoneurones could be synchronized over a time-scale of ± 20 to ± 50 ms (broad-peak synchronization). This was interpreted as reflecting presynaptic synchronization among inputs derived from signal cord interneurones. The question naturally arises whether the presynaptic synchronization reflected in the medium-width peaks could also influence motoneurone synchronization. This would certainly be the case if the monosynaptic connexions described here formed the majority of the motoneurone inputs. However, since we now believe this not to be the case (Davies *et al.* 1985), the influence of the synchronization in the medulla is less certain. It is possible that if the majority of the motoneurone inputs came from the bulbospinal neurones via

only one or two synapses, an effect could still appear. The sort of effect that we would expect would be a synchronization between motoneurones with a duration corresponding to about twice the duration of the medium-width peaks measured here, say ±5 to ±8 ms. This duration is wider than that of short-term synchronization (Sears & Stagg, 1976), but is narrower than the broad peaks measured by Kirkwood *et al.* (1982*b*).

There was some anecdotal evidence for such an effect. For instance, the animal which gave the medium-width peak shown in Fig. 7*B* gave similar peaks in most segments for each of the three bulbospinal neurones tested, and was thus extreme in the proportion of medium-width peaks present. The motoneurone synchronization in this animal was also extreme, being characterized by a peak with a high value of k (1·31 for motoneurones of adjacent segments) and a half-width of 6 ms. Such a peak corresponds exactly to the expectations set out above and, with hindsight, corresponds to a few of the peaks seen previously by Kirkwood *et al.* (1982*b*). They were then classified as strong short-term synchronization (perhaps with some weak broad-peak effects) but we now suggest that they had their origin in synchronization of inspiratory neurones in the medulla. Possible examples are those of fig. 4*F* of Kirkwood *et al.* (1982*b*), or fig. 3 (○) of Kirkwood *et al.* (1982*a*). The general conclusion to be drawn from these observations is that only the narrowest of peaks in short-term synchronization measurements on motoneurones (no wider than ±3 ms at the base) may be assumed to originate only from the actions of branched axons synapsing on both groups of motoneurones as was hypothesized by Sears & Stagg (1976). Even this criterion may be too generous.

Conduction velocities of bulbospinal neurones

The mean value of conduction velocity measured here (51·5 m/s) is greater than most values in the literature, in particular those from Bianchi (1971), Berger (1977) and Lipski, Trzebski & Kubin (1979). However, it is gratifyingly close to the value published recently by Dick & Berger (1985), who used more accurate methods (spike-triggered averaging of the orthodromic spike or two-point microstimulation). The results here are also accurate, partly because the method chosen depended on the orthodromic component of the collision interval and partly because the conduction distances were relatively long and therefore small errors in conduction time were not important. It is worth noting that although for the cervical and upper thoracic levels the conduction velocities were greater than those measured by Bianchi (1971), Berger (1977) or Lipski *et al.* (1979), because of the observed slowing the values here for the middle and lower thoracic levels are nearly identical to their values (32·7 m/s here, 31·5, 28·0 and 32·7 m/s for the others).

The transmission delays measured for the cross-correlation histogram peaks for the phrenic and the upper intercostal activities were the same, which indicates that even if axons descending to the thoracic levels give off collaterals to the phrenic nucleus, these axons do not show appreciable slowing of conduction at this point. It is possible, but not likely, that the population of units studied here is only a subgroup of those which are more frequently identified with a spinal stimulus at cervical levels. We think it unlikely because when the recording electrode was located properly in either the dorsal or ventral respiratory groups (i.e. among 'large' cells, with unit spikes recordable over a track length of typically 0·1 mm), most of the units could be

activated from the thoracic cord. Our experience therefore suggests that most inspiratory bulbospinal neurones of either group have axons extending as far as the thorax, contrary to an earlier report (Bianchi, 1971).

Conclusions

A monosynaptic pathway exists from the respiratory cell groups of the medulla to both phrenic and external intercostal motoneurones. We have defined a population of narrow peaks in a set of cross-correlation histograms which are used in the following paper (Davies *et al.* 1985) to attempt a quantitative assessment of the strength of this projection.

The medium-width peaks are also, via the model, consistent with the monosynaptic pathway, but on their own they are not strong evidence for it. This conclusion is similar to that deduced from h.f.o. some years ago (Cohen, 1973). Whether or not the medium-width peaks will prove useful as indicators of functional connectivity depends on how successfully they can be separated from h.f.o., how successfully presynaptic synchronization can be separated from oligosynaptic effects and also whether the synchronization of neurones within the medulla can be shown to have any functional significance. However, the separation of the medium-width peaks from h.f.o. may turn out to be, in general, a vain hope: it is conceivable that both forms of cross-correlation arise from the same set of connexions, perhaps an aperiodic common input which partially synchronizes the bulbospinal neurones, such synchronization becoming h.f.o. when a large proportion of the neurones, as seemed to be the case here, have a natural firing frequency in the h.f.o. range, 60–120 Hz.

APPENDIX

The calculations below were made to compare the time courses of peaks in cross-correlation histograms derived from the two situations illustrated in Fig. 11, namely a simple monosynaptic connexion (upper diagram) or the situation where the reference neurone in the medulla (A) does not itself excite the motoneurone (B) but is synchronized with a number of other neurones in the medulla (C_1 to C_n) which each do make that connexion monosynaptically. In addition, the equivalent time courses of spike-triggered average e.p.s.p.s were compared for the same two situations.

For the monosynaptic connexion, the e.p.s.p. time course, $f(t)$, in Kirkwood & Sears (1978) was assumed:

$$f(t) = K\{[(\beta-\alpha)t-1]\exp(-\alpha t)+\exp(-\beta t)\},$$

where K is a constant, t is time, $1/\beta$ is the time constant of decay of the e.p.s.p. (taken as 5 ms) and α is a variable which determines the rise time of the e.p.s.p. The e.p.s.p. for $\alpha = 20\beta$ is illustrated in Fig. 11 (top right).

The primary correlation kernel for a monosynaptic connexion, $f'(t)$, is the time course of the raised probability of firing of the motoneurone created by this e.p.s.p. and was calculated from:

$$f'(t) = af(t)+b\frac{\mathrm{d}f(t)}{\mathrm{d}t}.$$

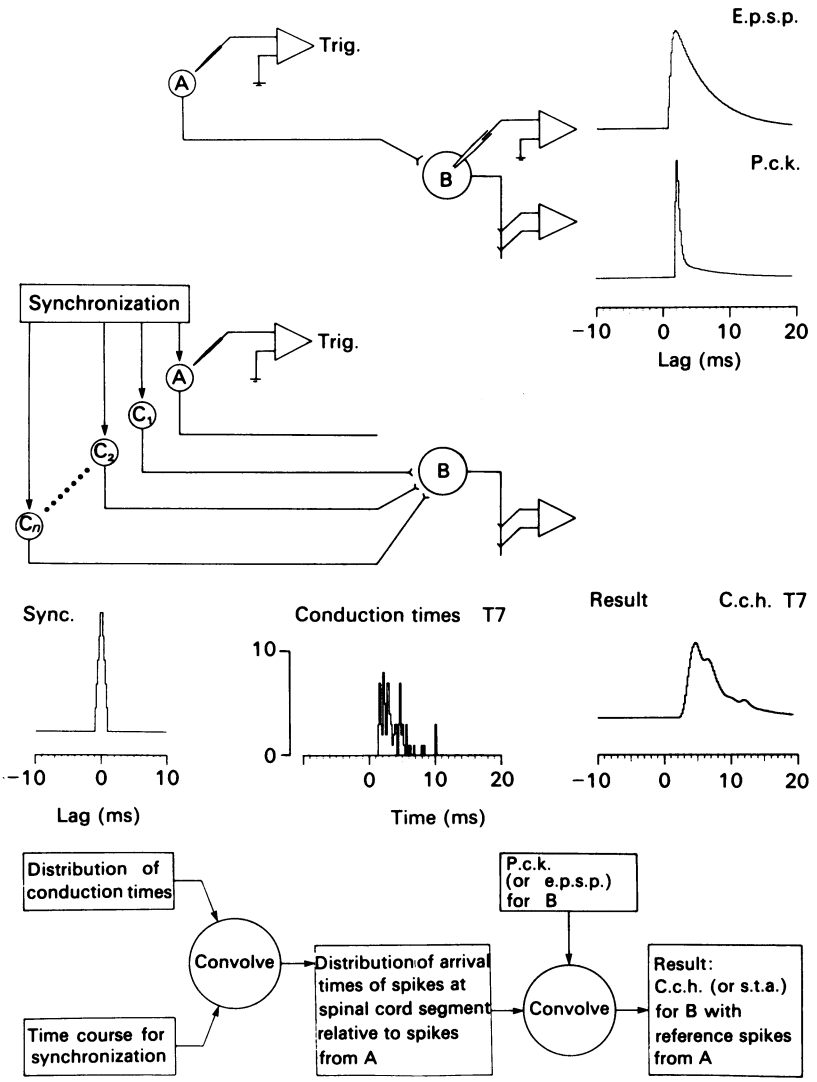

Fig. 11. Scheme for theoretical estimation of the time courses of medium-width peaks. Top, ideal situation for spike-triggered average or cross-correlation experiments: a neurone, A, fires independently of others and monosynaptically excites a motoneurone, B. Spike-triggered averaging (s.t.a.) of synaptic noise in B gives the e.p.s.p., while cross-correlation between the neurone and motoneurone discharges gives the equivalent primary correlation kernel (p.c.k.). Middle, assumed situation where the neurone providing the trigger spikes, A, is partially synchronized with other neurones (C_1 to C_n) which excite the motoneurone, B, monosynaptically, each one giving an e.p.s.p. as above. The time course of synchronization between *A* and (C_1 to C_n) is shown (Sync.) together with the distribution of conduction times in their axons for a distance corresponding to medulla – T7. The resulting cross-correlation histogram (c.c.h.) from the discharges of A and B is shown at the bottom right and the computational scheme used to produce this result is shown below. See text for more details.

The example shown in Fig. 11 (primary correlation kernel, p.c.k.) is for $\alpha = 20\beta$ and $b/a = 0{\cdot}1$ ms. (This corresponds to $b/a = 0{\cdot}5$ in Kirkwood & Sears, 1978.) The same e.p.s.p. or p.c.k. was assumed for the monosynaptic connexions between A and B in the first diagram and between C_1 to C_n and B in the second.

The cells in the medulla (A, C_1 to C_n) were assumed to be synchronized by an undefined aperiodic mechanism with a time course given by a symmetrical, triangular raised probability of firing with a maximum at time zero, as is shown in Fig. 11 (Sync.). The illustrated time course is what would be seen in a cross-correlation histogram between the discharges of any pair of these neurones. Neurones C_1 to C_n are assumed to have the distribution of conduction velocities which was measured experimentally and is shown in Fig. 2*A*. For any given spinal segmental location of motoneurone B, a corresponding distribution of conduction times from medulla to motoneurone was calculated, on the assumption of standard distances, which themselves corresponded to the average-sized cat (about 3 kg) in our sample, namely distances of 59, 102, 122, 143 and 164 mm for segments C5, T3, T5, T7 and T9 respectively. This distribution is shown in Fig. 11 for T7. An additional fixed time delay (1·5 ms) corresponding to the mean transmission delay estimated in this study was also assumed for the cross-correlation histograms. A corresponding delay of 1 ms, appropriate to slowing in segmental presynaptic branches plus synaptic delay, was assumed for the spike-triggered averages.

We based the most important assumption, that neurones covering the observed range of conduction velocities (C_1 to C_n here) converge onto the motoneurone B, on the relative ease of detecting synchronization in the medulla (op. cit.). However, even if that justification is doubted for a given motoneurone on the grounds of limited convergence, the assumption may still be considered valid for the cross-correlation histograms of this study, which were derived from multi-unit data. The relatively large number of motoneurones involved may quite reasonably be assumed to 'sample' all these descending fibres.

The calculations are simple (Fig. 11, bottom). The convolution of the triangular time course of synchronization for the medulla with the distribution of conduction times gives a distribution of arrival times of synchronized presynaptic spikes in a given segment with respect to spikes of the reference unit *A*. The convolution of that distribution of arrival times with the primary correlation kernel, $f'(t)$, gives the predicted time course of the cross-correlation histogram (c.c.h.) between *A* and *B*. This is illustrated in Fig. 11 (c.c.h. T7) for the same parameters as the rest of the Figure. An equivalent spike-triggered average in cell B (not illustrated in Fig. 11) is given by the same process but using the monosynaptic e.p.s.p., $f(t)$, in place of $f'(t)$.

We retained $b/a = 0{\cdot}1$ ms for most of the calculations on the basis of the experimental results of Kirkwood & Sears (1978) and Gustafsson & McCrea (1984) and have chosen $\alpha = 20\beta$ as the most likely value of this parameter in $f'(t)$ (as in Fig. 11), because this is not only close to the best fit of Kirkwood & Sears (1978) but also gives a half-width of the primary correlation kernel of 0·7 ms, very close to the observed half-widths of the narrow peaks here. The most likely value for the time span of the synchronization in the medulla was chosen to be ± 1 ms (at the base of the peak) after inspection of published records (op. cit.).

In order to show some of the variations possible in the model we also chose a set

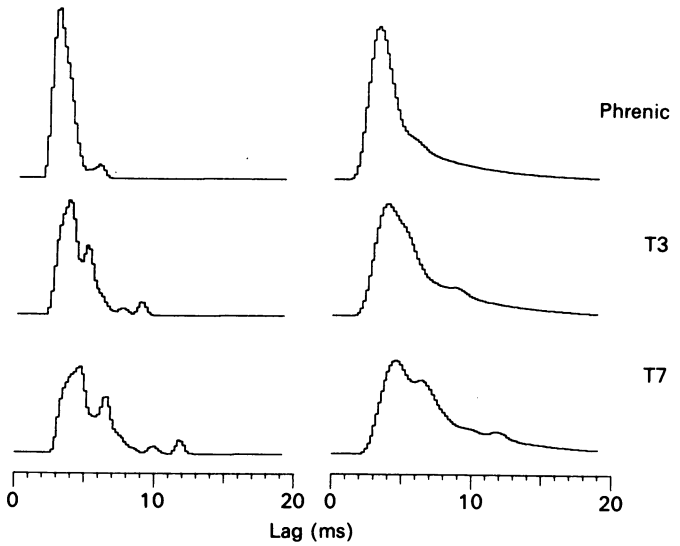

Fig. 12. Theoretical time courses of medium-width peaks for motoneurones at three different segmental levels. Left column, short durations assumed for synchronization in the medulla ($\pm 0{\cdot}4$ ms) and for the primary correlation kernel ($b/a = 0{\cdot}2$ ms, $\alpha = 50\beta$). Right column, the 'most likely' values assumed for synchronization in the medulla (± 1 ms) and for the primary correlation kernel ($b/a = 0{\cdot}1$ ms, $\alpha = 20\beta$).

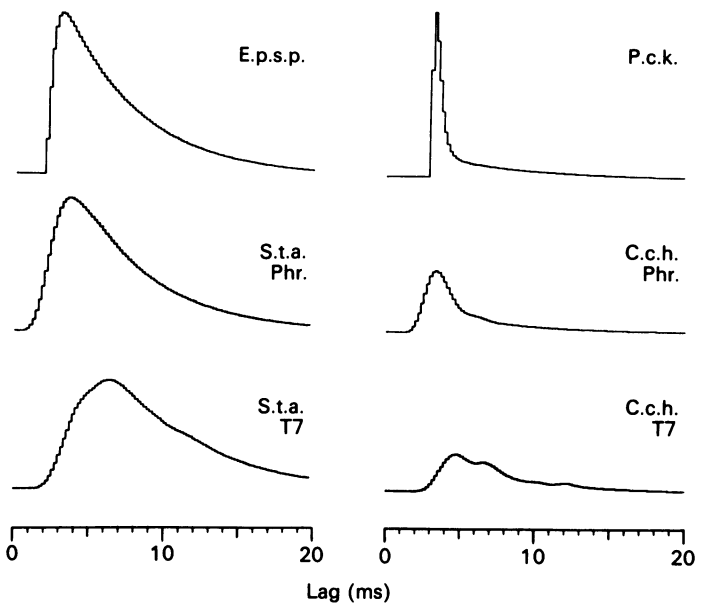

Fig. 13. Comparisons of theoretical medium-width peaks for phrenic and T7 intercostal motoneurones (c.c.h. Phr., c.c.h. T7) with theoretical spike-triggered averages of synaptic noise (s.t.a. Phr., s.t.a. T7) derived from the same degree of presynaptic synchronization ('most likely' values assumed; e.p.s.p. and primary correlation kernel as illustrated).

of parameter values which would give shorter durations. They were: for $f'(t)$, $b/a = 0{\cdot}2$ ms (this gives the peak in the primary correlation kernel *only* during the rising phase of the e.p.s.p.) and $\alpha = 50\beta$; for the time span of the medullary synchronization, $\pm 0{\cdot}4$ ms.

The predictions from these two sets of parameter values are shown in Fig. 12 for various segmental levels, for the most likely values in the right-hand column and the other values in the left-hand column. The time courses on the left are determined almost entirely by the distribution of conduction times, whereas the durations of the primary correlation kernel and the synchronization in the medulla make a more noticeable contribution on the right. The uneven, bumpy shapes, particularly on the left, are simply due to using a relatively unsmooth distribution of conduction velocities (Fig. 2*A*).

Fig. 13 shows comparisons between average depolarizations due to presynaptic synchronization and medium-width peaks, predicted using the 'most likely' parameter values. It should be noted that although the vertical scales are arbitrary they are equivalent for the average depolarization and cross-correlation histogram. In each case the same number of contributing axons has been assumed: the areas under the average depolarization and the component monosynaptic e.p.s.p.s have been made equal as have the areas under the cross-correlation kernel and the medium-width peaks. It should be noted that for C5 (i.e. for phrenic motoneurones) the average depolarization is 0·82 times the amplitude of the monosynaptic e.p.s.p., whereas the medium-width peak in the cross-correlation histogram is only 0·38 times the amplitude of the primary correlation kernel.

We are grateful for support provided by the Brain Research Trust and the endowment funds of the National Hospital for Nervous Diseases.

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