

The incidence of initial doublets in the discharges of motoneurons of two different inspiratory muscles in the cat

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1. Trains of action potentials in motoneurons frequently commence with an initial doublet; i.e. a uniquely short interspike interval. Previous authors have speculated on the functional importance of initial doublets. Here we test the hypotheses that these doublets are associated with particular classes of motoneurons or particular physiological conditions.
2. Discharges of inspiratory motoneurons were recorded extracellularly in the thoracic ventral horn of anaesthetized, paralysed cats. Seventy units (35 each with axons in the internal and external intercostal nerves) were classified on the basis of their maximum firing rates, start times in the respiratory cycle and axonal destination.
3. Initial doublets were defined by an interspike interval <14 ms. Of seventeen units firing initial doublets, fifteen had axons in the external intercostal nerve and two had axons in the internal intercostal nerve. Neither maximum firing rate nor start time during the respiratory cycle predicted the occurrence of doublets.
4. The chemical drive to breathe was manipulated by altering the CO₂ content of the inspired gas or by briefly stopping the respiratory pump. Varying the chemical drive to breathe had no consistent effect on the occurrence of initial doublets.
5. These results support the view that initial doublets are part of the normal pattern of discharge of motoneurons. However, because the incidence of doublets does not consistently support previous functional hypotheses, we argue that the occurrence of doublets may not necessarily be dictated by the CNS, but in some circumstances it is an epiphenomenon dependent on the state of the motoneuron, in particular on the statistical properties of its synaptic inputs.

It has been known for more than 60 years that mammalian motoneurons can sometimes generate pairs of impulses (doublets) with an unusually short interval compared with the current mean rate of discharge (Adrian & Bronk, 1929; Denny-Brown, 1929; Eccles & Hoff, 1932). Doublets occurring at the very start of an impulse train (initial doublets) are the most common. Despite the long history of their recognition, there is no consensus on the mechanism for their generation, on their physiological role, or indeed on their manifestation in natural movements (Bevan, Laouris, Reinking & Stuart, 1992).

For instance, initial doublets (sometimes triplets) occur during treadmill or fictive locomotion in decerebrate cats (Severin, Shik & Orlovsky, 1967; Zajac & Young, 1980; Jordan, 1983). These doublets have an instantaneous frequency of 50–300 Hz, and are followed by a longer interval prior to the firing of the motoneuron at a fairly constant 'preferred discharge rate' (Zajac & Young, 1980). It was observed that 80% of both flexor and extensor motoneurons fired such doublets in the majority or all of

their bursts. Zajac & Young (1980) suggested that these doublets play a role in the regulation of muscle tension, and that the subsequently reduced rate is related to a build-up of motoneuron after-hyperpolarization after the first spike. However, Hoffer, Sugano, Loeb, Marks, O'Donovan & Pratt (1987) recorded motoneuron spike trains from awake intact cats during treadmill locomotion and reported that initial doublets were but rarely seen (4 of 51 motoneurons). They attributed the difference in results to some 'systematic change introduced by decerebration', for example 'long-lasting changes in the intrinsic membrane properties of spinal motoneurons' resulting from decerebration (p. 551, Hoffer *et al.* 1987).

Similarly, Bawa & Calancie (1983) were able to record trains of doublets in a high proportion of their own (healthy) motor units in flexor carpi radialis (see also Kudina & Churikova, 1990; Kudina & Alexeeva, 1992), whereas some previous authors (see Bawa & Calancie, 1983 for refs) had taken the occurrence of doublets in human EMG recordings as being rare or pathological.

In awake rats Hennig & Lomo (1987) found doublets only in extensor digitorum longus units, deduced from their other firing patterns to be fast fatigable units. They suggested that the occurrence was related to the need to produce extra tetanic force in the strong contractions in which these units would be recruited, although they noted that some slower soleus units could also show doublets after section of the nerve to antagonists (Hennig, 1987). In contrast, Spielman, Laouris, Nordstrom, Robinson, Reinking & Stuart (1993) claimed that it was type S motoneurons that were most likely to fire doublets when excited by extracellularly applied currents.

In respiratory motoneurons initial doublets have been seen only under some circumstances, including respiratory, but not head rotation movements for human sternocleidomastoid (Adams, Datta & Guz, 1989), asphyxia for rabbit external intercostal and phrenic motoneurons (Macefield & Nail, 1987) and asphyxial gasping for cat laryngeal motoneurons (Davis, Macefield & Nail, 1987). Frize (1970) analysed the firing of three external intercostal motoneurons recorded in anaesthetized cats in this laboratory (T. A. Sears & D. Stagg, otherwise unpublished data) and all showed initial doublets. However, these last recordings were made from peripheral nerve branches, where the discharges of other units allow only the lowest threshold units to be studied.

In the present experiments we have re-investigated the matter by using extracellular microelectrodes, which enable single units to be distinguished at any level of activity, and have recorded the firing of individual inspiratory motoneurons in the thoracic ventral horn. Firstly, we have confirmed, in cats with an intact neuraxis, that doublets can be part of a 'normal' pattern of discharge and secondly we have shown that during this pattern only one out of two groups of motoneurons show doublets, this distinction being different from any of the other distinctions mentioned above. This leads us to suggest, *inter alia*, that the occurrence of doublets may not always be functionally important.

A preliminary account of this work has appeared (Kirkwood, Munson & Westgaard, 1988*b*).

METHODS

The observations come mostly from experiments already reported (Kirkwood, Munson, Sears & Westgaard, 1988*a*; Schmid, Kirkwood, Munson, Shen & Sears, 1993) (21 cats), but additional data have come from another five cats where the same basic procedure was followed. The cats (adult, either sex) were anaesthetized with sodium pentobarbitone (initial dose 37.5 mg kg⁻¹ i.p., then i.v. as required), paralysed with gallamine triethiodide (subsequent to surgery) and artificially ventilated, via a tracheal cannula, with oxygen-enriched air in order to bring the end-tidal CO₂ fraction initially to about 4%. CO₂ was then added to the gas mixture to raise the end-tidal level to a value sufficient to give a brisk respiratory discharge in the mid-thoracic intercostal nerves (typically 6–7%). In some animals the inspired fraction of

CO₂ was later varied in order to alter the respiratory drive without changing the mechanical conditions. Anaesthesia was assessed by continuous observations of the patterns of the respiratory discharges and the arterial blood pressure (measured via a femoral cannula) together with responses, if any, of both of these to a noxious pinch of the forepaw. Only minimal, transient, responses were allowed before supplements (5 mg kg⁻¹) of pentobarbitone were administered. The animal was mounted, prone, by vertebral clamps typically at T4 and T10 and a clamp on the iliac crest. The head was supported. Rectal temperature was maintained between 37 and 38 °C by a heating blanket. The bladder was emptied manually at intervals. The animals received occasional infusions of 5% Dextran in saline and their systolic blood pressures were above 100 mmHg throughout.

Various thoracic nerves were prepared for stimulation or recording via platinum wire electrodes. The following nerves were relevant for the present measurements. Firstly, the internal intercostal nerve and a bundle of dorsal ramus nerves on the left side of one segment (T1 to T6) in which recordings with an extracellular microelectrode were to be made. These nerves were used for antidromic stimulation, the field potentials from which were used to locate the microelectrode (Kirkwood *et al.* 1988*a*). Secondly, the internal intercostal nerve and the external intercostal nerve of that segment were used for recording spike-triggered averaged efferent spikes (see below). Thirdly, discharges from the external intercostal nerve, or those from the external intercostal nerve of a more rostral segment, were used to define the timing of central inspiration.

A thoracic laminectomy of several segments (Kirkwood *et al.* 1988*a*) was made, the dura opened and small patches of pia removed from the dorsal columns of the segment to be investigated. Except in the more rostral segments (Kirkwood *et al.* 1988*a*) a shaped pressure plate was lightly applied to the cord dorsum of the segment of interest in order to limit movements and hence maintain recording stability. The spinal cord and nerves were submerged in a single paraffin oil pool constructed from skin flaps. A glass microelectrode (either broken to about 3 µm external tip diameter and filled with 3 M NaCl, or filled with 3 M NaCl and bevelled to a resistance of 3–20 MΩ) was inserted into the left side of the spinal cord via a hole in the pressure plate, using an angle of 15 deg to the vertical in the transverse plane. From this electrode recordings were made of spike trains from inspiratory motoneurons whose axons lay in either the external or internal intercostal nerve, as identified by spike-triggered averaging from one or other of these nerves (Fig. 1*C*). The recordings were displayed on an oscilloscope and fed to a loudspeaker.

The motoneuron spike trains and efferent nerve discharges for a variable number of respiratory cycles (depending on the procedure for that particular cell, e.g. see Kirkwood *et al.* 1988*a*) were stored on magnetic tape for later analysis. For some units attempts were made to change the proportions of doublets by varying the level of motoneuron excitation via changes in the chemical drive to breathe. Either the CO₂ content of the inspired gas was raised or lowered to a new steady state or the respiratory pump was switched off for a brief period to produce a steadily increasing hypercapnic hypoxia. In each case either a number of respiratory cycles were recorded on tape at each of several steady states or a continuous recording was made as the state was changed. Successive intervals in the motoneuron discharge were measured by computer to an accuracy of 1 ms. The maximum firing rate (in impulses per second) of each unit for each cycle for a given

condition was estimated via a spike counter, as in Kirkwood *et al.* (1988*a*), from the mean maximum count per cycle in 200 ms bins from five or ten successive cycles. The start time in any cycle was estimated as the time of occurrence of the first spike as a proportion of inspiratory time (T_I), as defined by the α -motoneurone discharges in most active external intercostal nerve recording, again as in Kirkwood *et al.* (1988*a*).

RESULTS

Identification of doublets

Doublets were usually readily detected by ear or on the oscilloscope. An example of an early recruited unit with a particularly prominent occurrence of doublets is shown in Fig. 1*A*. Two selected inspirations are shown, the first one

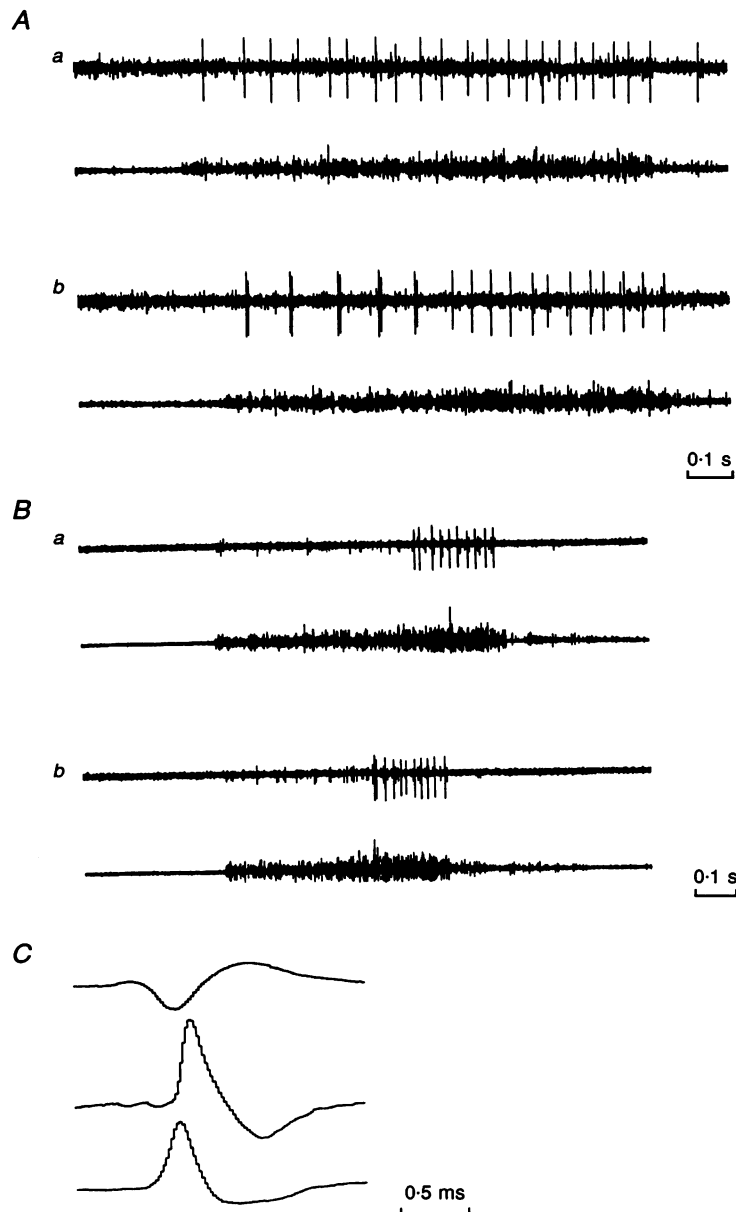


Figure 1. Examples of motoneurone recordings

A and *B*, discharges during the inspiratory phase of two different respiratory cycles (*a* and *b*) for each of two different external intercostal motoneurones. *A*, an early-recruited example; *B*, a late-recruited example. The discharge of the external intercostal nerve, containing the axon of the illustrated motoneurone, is shown underneath the single-unit recording in each case. *C*, spike-triggered averaging for the identification of a motoneurone. The middle trace is the average of 256 sweeps of the external nerve recording, triggered by the single-unit spikes, whose timing is shown by their own average (32 sweeps) in the top trace. The bottom trace is another average from the external intercostal nerve (64 sweeps, gain reduced by one-quarter), but triggered from the largest spikes in this nerve discharge (approximately 80–100% amplitude). Comparisons of the middle trace with the bottom trace give an approximate estimate of the relative axonal spike amplitude of the single unit.

without (*Aa*) and the second one with (*Ab*) doublets. As has been reported many times, the interval succeeding the abnormally short doublet interval was usually longer than subsequent single-spike intervals. Trains of doublets were sometimes seen, as the five successive pairs in Fig. 1*A* *b*, but they were uncommon. When they were seen, the usual pattern was a gradual lengthening of the doublet interval as the train progressed, coupled with a gradual shortening of the post-doublet interval, as has been previously reported (Frize, 1970; Kudina & Alexeeva, 1992). Most often only a single doublet was seen as the first discharge in inspiration, as in Fig. 1*B*. The unit in Fig. 1*A* also gave a few doublets as the second interval of an inspiratory burst (Fig. 2), the first interval being rather long, but was one of only three units to do so. One other unit gave some initial triplets or quadruplets and also some other doublets later in the burst, but for all other units only doublets were seen and for all but one of these the doublets occurred only at the start of an inspiratory burst.

The one remaining unit (from the external intercostal nerve) was unique in showing alternate short and long intervals for more than 50% of its intervals, of which about five per cycle were short enough to be considered doublets (see below). However none of them were initial doublets, so this unusual unit is excluded from the subsequent statistics. Another two external nerve units are also excluded from the statistics, despite giving initial doublets, because the external nerve discharge was unusually bursty in character at the time these units were recorded. The doublets were clearly associated with the bursts as much as with the cycle as a whole and it is therefore not appropriate to compare these with those associated with the normal, relatively smooth profile of the inspiratory discharges. More generally, the

proportion of cycles in which a unit gave a doublet could be anywhere from 0 to 100%.

The unit in Fig. 1*A* was an early-recruited, slow-firing one. By contrast Fig. 1*B* shows the firing of a late-recruited, fast-firing one. This unit also fired doublets, as is clear in the second of the two cycles illustrated (Fig. 1*B* *b*). The first cycle (Fig. 1*B* *a*) also starts with a rather short interval, followed by a longer one, but, since the preferred firing rate of the unit is already high, it is less clear whether or not this cycle should be counted as one with a doublet. The procedure illustrated in Figs 2 and 3 was therefore followed to clarify the situation.

The procedure consisted of plotting the duration of the second interval against that of the first for each inspiratory burst. Figure 2 represents this for the unit of Fig. 1*A*. The situation is very clear here: ignoring the few points near the abscissa, which represent doublets following the second spike, the remainder fell into two groups, those on the left representing cycles with doublets, those scattered near the line of equality representing those without doublets. Another general feature is that in the non-doublet group there are more points below the line of equality (38) than above (13). This represents the well-known, generally incrementing form of the inspiratory efferent discharges. However, because of this, the significance of our earlier statement that the intervals succeeding a doublet were abnormally long could be questioned; i.e. were these intervals longer than the subsequent ones merely because of the inspiratory ramp? Figure 2 shows that in fact this is unlikely to be the only explanation. The second intervals from the doublet group (median 108 ms) are generally longer than either the second intervals (median 78 ms) or the first (median 93 ms) from the non-doublet group.

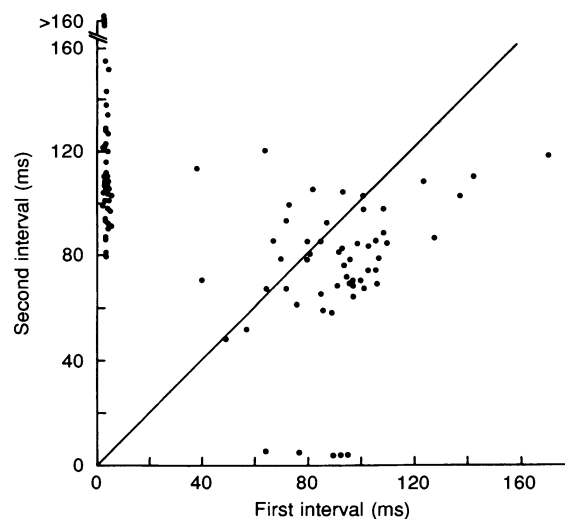


Figure 2. Identification of doublets

Plot of the second interval of each inspiratory burst against the first interval, for the unit in Fig. 1*A*. The group of points near the ordinate represent cycles with initial doublets. The remaining points represent those without. Line of identity drawn in.

Similar plots from three other units showing doublets, but covering a wide range of firing frequencies, are shown in Fig. 3*A* and three more examples roughly matched with these for firing frequency, but without doublets, are shown in Fig. 3*B*. For either plot the identification of cycles with or without doublets is obvious for the two slowest-firing units, but much less so for the fastest-firing one. The fastest-firing one in Fig. 3*A* (the unit of Fig. 1*B*) was classified as showing doublets because it showed first intervals in the same range as the other units which unequivocally showed doublets (2–14 ms), whereas the fastest-firing one in Fig. 3*B*, whose first intervals were all 14 ms or above was classified as not showing them. In the analysis that follows, individual cycles were classified as showing a doublet if the first interval was < 14 ms. The dividing line here is clearly rather arbitrary, but all the other units showed a clearer distinction than these two (cf. Figure 5*B*, the next most ambiguous case).

In Fig. 3*B* only the slowest firing unit showed the second interval as generally shorter than the first, i.e. evidence of

the ramp. This may have been because both of the other two were recruited late in inspiration (55 and 75% of inspiratory time), when the ramp was often less prominent.

Which units showed doublets?

Units were classified by maximum firing rate and by start time, both of which should be related to recruitment order and motor unit type (see Discussion) and also by the axonal destination. Of these three, only the axonal destination appeared to be related to the propensity of the unit to give doublets. All but two of the seventeen units firing doublets were external intercostal nerve units. The proportion of external nerve units showing doublets (15/35) was highly significantly different from that for the internal nerve (2/35; χ^2 test, $P < 0.001$).

This is illustrated in Fig. 4, which also shows that the external nerve units giving doublets (shaded bars) were not concentrated in any part of either the start time or firing rate distributions. Moreover, there did not appear to be any relation between the proportion of cycles giving doublets

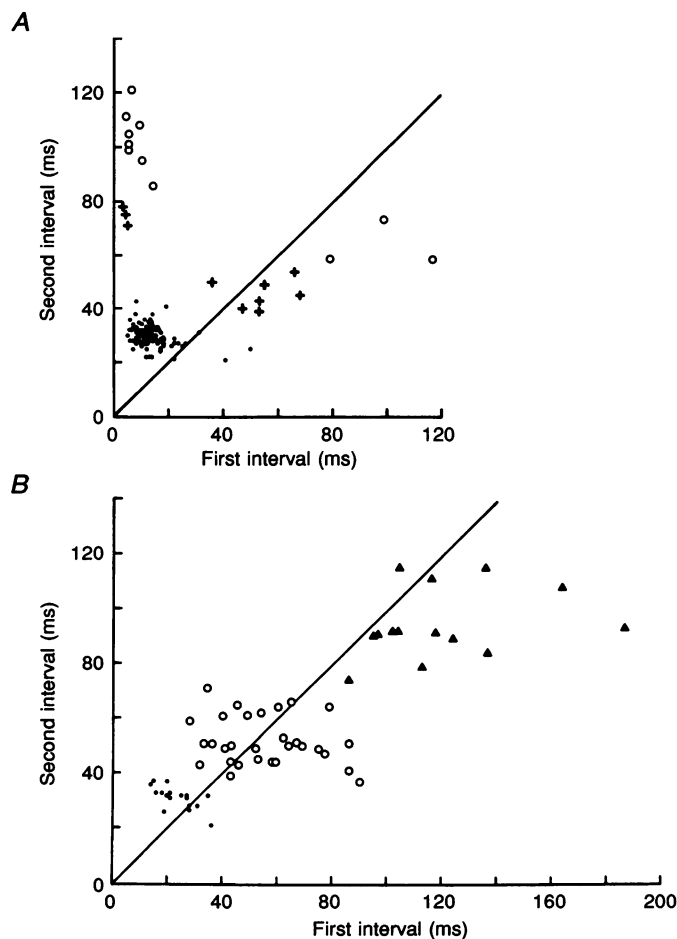


Figure 3. Comparison between units giving and not giving doublets

A, plots as Fig. 2 for three external nerve units giving doublets (different symbols for each unit). *B*, similar plots for three different units not giving doublets; O, from the external nerve; ● and ▲, from the internal nerve. The typically clear grouping of points for the two units giving the longer second intervals in *A*, as in Fig. 1, leads to the definition here of a doublet as a pair of spikes separated by less than 14 ms.

(intensity of shading) and either of these two variables. Thus, units showing doublets were not restricted to fast-firing late-recruited units as in Henning & Lomo (1987). Further, since both early- and late-recruited units showed doublets, it is clear that the occurrence of doublets was not restricted to any particular time in the respiratory cycle.

Figure 4 also shows that the distributions of start time and firing rate were very similar for the external nerve (A) and for the internal nerve (B) and confirms quantitatively what also seemed clear by qualitative inspection, that the gross firing patterns of the units from the two nerves were not different. This suggests a similar gross pattern of synaptic drive to motoneurons of the two nerves and a similar distribution of motor unit types in the two nerves. Thus the difference in doublet incidence between the motoneurons of the two nerves is unlikely to be due to a systematic difference in either of these factors.

One of the internal nerve units in Fig. 4 looks like it may be part of a different population from the rest, having a much higher maximum firing frequency (107 impulses s^{-1}) than the others. This particular unit was probably a γ -motoneurone, as indicated by the amplitude and latency of its nerve spike in the spike-triggered average. Only two other units, both from the external nerve, (maximum firing rates 39 and 59 impulses s^{-1}) were suspected of being γ -motoneurons by the same criteria. However, the identification was uncertain, partly because the criteria are only approximate. The criterion of spike amplitude (relative to the largest amplitude units in the nerve; cf. Sears, 1964a) is not really quantitative and the latency can only give an approximate

estimate of conduction velocity because of the short conduction distance and the considerable uncertainty of the timing of the extracellularly recorded unit spike with respect to the motoneurone axon spike. The uncertainty of identification is greater for γ -rather than α -motoneurons because even if units had well-identified small diameter axons they could still have been α -motoneurons whose axons had divided within the muscle nerve (Eccles & Sherrington, 1930). It is therefore unlikely that more than these three units were γ -motoneurons. If the suspected γ -motoneurons are omitted, the proportions of units firing doublets for the two nerves (14/33 vs. 2/34) are still significantly different ($P < 0.01$). It should be noted also that it is in any case most unlikely that this difference resulted from a larger proportion of γ -motoneurons being present in the internal nerve than in the external, since the only inspiratory muscle innervated by the internal nerve, the parasternal interchondral muscle, has been reported to have very few spindles (Duron, Jung-Caillol & Marlot, 1978). A number of other probable γ -motoneurons were also recorded, but they were either external nerve units or expiratory internal nerve units and they all fired continuously throughout the respiratory cycle, as is common for intercostal γ discharges (Sears, 1964a). Because of their continuous firing patterns these units could never show initial doublets and they were excluded from the analysis.

Synaptic drive and incidence of doublets

For most units showing doublets, the respiratory cycles with doublets seemed to occur at random with respect to cycles without doublets. However, occasionally a unit appeared to switch between these two modes of firing for relatively prolonged periods. For instance, the one in Fig. 1A, with an overall incidence of 41/91 cycles with doublets, showed

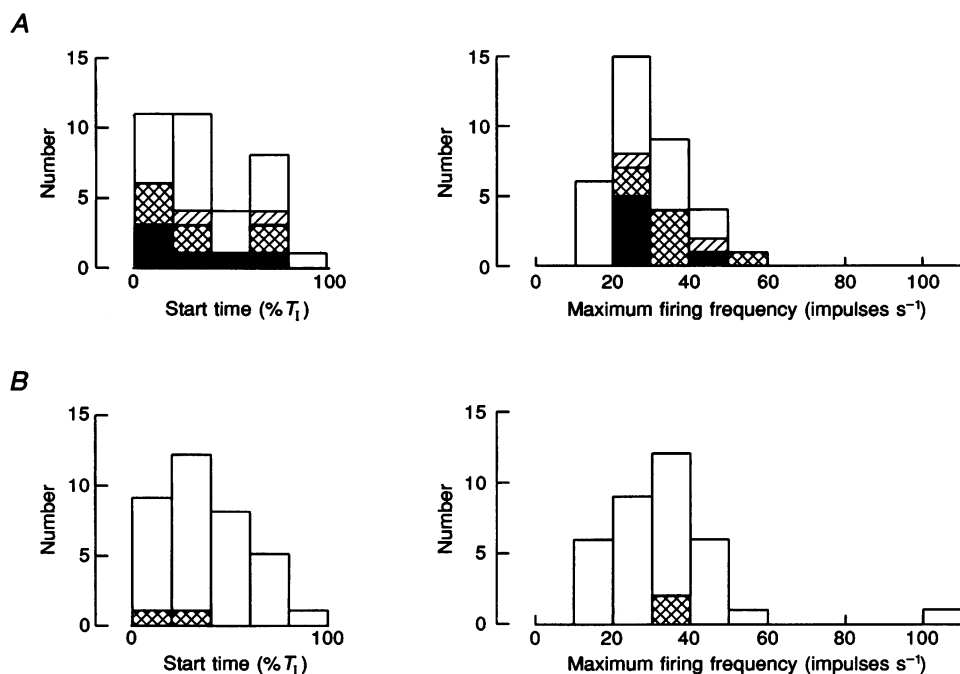


Figure 4. Comparison between external (A) and internal (B) intercostal nerve units

Start times and maximum firing frequencies are shown for all the units, classified by the proportion of respiratory cycles showing doublets for each (\square , 0%; diagonal lines , 1–25%; cross-hatch , 26–75%; and \blacksquare , 76–100%). Doublets are clearly more common for the external nerve units than for the internal nerve, and occur through most of the ranges of both start times and firing frequencies.

trains of up to 10 cycles with doublets and trains up to 17 cycles without. It therefore appears that doublet firing could be dependent on some state of either individual units or of the preparation. One factor in that state could have been the overall level of synaptic excitation of the motoneurone.

This could have had two possible effects on the incidence of doublet firing. Firstly, an increased excitation might be expected to lead to a higher incidence of doublets, via the teleological arguments of, among others, Hennig & Lomo (1987), which relate the occurrence of doublets to the firing of high threshold units and the extra tetanic tension that doublets can produce. Secondly, and conversely, Bawa & Calancie (1983) produced doublets in the motoneurones of their own forearm muscles by maintaining firing levels rather close to threshold, representing in the present experiments a level of synaptic drive reduced from the rather strong respiratory drive which was routinely employed.

The first way this issue was addressed was, for those units showing a variable incidence of doublets, to relate the level of drive on a cycle-by-cycle basis to the incidence of doublets, as illustrated in Fig. 5A. This shows, for four different units, the number of doublets per cycle (all initial doublets, as in Fig. 1) plotted against an index of the level of excitation in each cycle, the total number of spikes per cycle. These plots give some support to the first suggestion, in that there were slightly more doublets for cycles with more spikes in total, but the effect was not at all strong.

The matter was therefore also investigated by producing larger changes in synaptic drive experimentally, by changing the chemical drive to breathe. The plot with the clearest dependency of doublets on excitation (the bottom plot in Fig. 5A, which represents the unit of Fig. 1A) comes from such an experiment. The cycles represented in Fig. 5A were drawn both from a control run at an end-tidal CO_2 level of 7% and a run at around 5.5%, where the unit was very close to its threshold for firing.

Another twelve units (8 from external, 4 from internal nerves) in four cats were subjected to a similar test (CO_2 levels reduced until the unit just stopped, or almost stopped firing). Only four of these fired doublets, one with an incidence of 100% and three with only a very low incidence. No increase in incidence was seen near threshold for any unit and for those firing doublets the incidence of doublets either stayed at 100% or was too low to be able confidently to detect any decrease.

For twelve units (8 the same as above; 9 external, 3 internal) tests were also made with an increased level of CO_2 . For five of these a new steady level was imposed, for six of them the pump was switched off and the firing was observed as the CO_2 (and O_2) levels changed, and for the remaining unit both were done. Eleven of the units gave no or very few doublets in the control state and no change was observed with the increased drive. The firing of the other unit is illustrated in Fig. 5B.

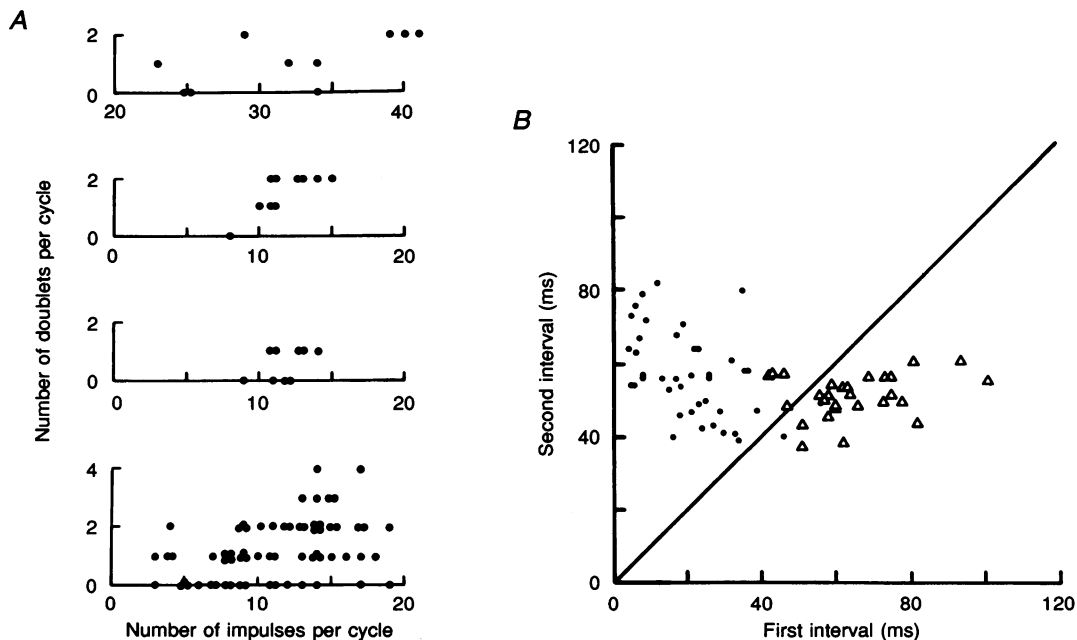


Figure 5. Variability in the incidence of doublets

A, plots for four different units of the number of doublets per cycle against the total number of impulses per cycle. B, plot as Fig. 2 for a unit where the proportion of cycles showing doublets changed with the state of the preparation. Symbols represent: ●, 7.5%; △, 8.9% end-tidal CO_2 , respectively. See text for explanation.

This unit was the only one where an imposed procedure produced a clear change in doublet incidence. The increase in the CO₂ level from an end-tidal value of 7.5 to 8.9% was accompanied by a change in the proportion of cycles showing doublets from 13/42 to 0/26. The graph (Fig. 5B) shows two sets of points with virtually no overlap for the two conditions. However, when the end-tidal CO₂ level was reduced to control levels the doublets did not reappear, although points plotted on the same graph for this condition (not shown) were somewhat to the left of those for the high level of CO₂.

DISCUSSION

The results here give no support for special significance to be put on the occurrence of doublets in terms of either unphysiological conditions or of the need for particularly strong contractions. The first of these conclusions depends on the assumption that the anaesthetized, paralysed cat produces a 'normal' pattern of discharge in its respiratory motoneurons. This must remain an assertion, although this pattern is, of course, only one of many patterns that these motoneurons can show. The direct effects of gallamine, or the effects of paralysis, can largely be ruled out as a cause of the doublets in the external nerve motoneurons because of the observations of Frize (1970), which related to nerve discharges in spontaneously breathing anaesthetized animals. Her results also control for the unlikely possibility that doublets could result from damage to the motoneurons by the electrode, as does the general absence of doublets for the internal nerve. In the present experiments we also observed doublets in recordings from branches of the external nerve (when the preparation permitted), but they were not studied systematically. For human recordings most authors now agree that doublets are part of normal patterns of discharge (Kudina & Churikova, 1990; Baker, Davey, Ellaway & Friedland, 1992).

As for the second of our conclusions, the relative independence of doublets from strong contractions, this is again in line with the human work (Bawa & Calancie, 1983; Kudina & Churikova, 1990; Kudina & Alexeeva, 1992). In the present experiments we have to exclude the use of a relatively high baseline level of CO₂ as itself promoting doublet firing. We found the high level of CO₂ useful in increasing the yield of respiratory neurons recorded. The results of Frize (1970), for which a eucapnic level of CO₂ applied, argue against this as a biasing factor, as do the few tests here where reduction of the CO₂ level did not eliminate existing doublets. Another possibility is that the high level of CO₂ might be accompanied by acidosis, which Hoff & Grant (1944) claimed was a potentiating factor in the decerebrate cat. We did not measure blood pH, but Denslow (1948), who studied this systematically in man for motoneurons from a variety of muscles, some of which gave a high proportion of units showing doublets, found no such an effect.

A possible alternative to the idea that a high intensity drive itself promotes doublets is that only high threshold motor unit types show them. Our conclusion, that this is unlikely for cat intercostals, is consistent with that of Cordo & Rymer (1982) and Bawa & Calancie (1983) but not with that of Hennig & Lomo (1987), though it is worth noting that in the study of Bawa & Calancie the units firing doublets were those with the fastest maximum firing frequencies to voluntary contractions. The association here of motor unit type with recruitment time and firing rate is indirect, but it is the same logic as used by Hennig & Lomo (1987). It should also be noted that the external intercostal muscle is known to contain motor units with a wide variety of mechanical properties (Andersen & Sears, 1964).

Our results also suggest that there is no need to postulate even strong *transient* synaptic inputs. External intercostal motoneurons usually receive a relatively smoothly rising depolarization during inspiration (Sears, 1964*b*; Kirkwood, Sears, Tuck & Westgaard, 1982) and the motoneurons here could show initial doublets at any time in this ramp. In particular the sudden offset of inhibition at the end of expiration (Sears, 1964*b*), which *a priori* might be thought to be a probable stimulus for doublets (Macefield & Nail, 1987), cannot be a major contributor. Of course we cannot rule this out as having caused *some* of the doublets here which occurred at the very start of inspiration. In the same way large transient depolarizations may have caused some of the occurrence of doublets for those units where the nerve showed bursty discharges and which were excluded from the main analysis. However, even if large transient events are *sufficient* to cause doublets they are clearly not necessary.

Instead of any of the above hypotheses we wish to suggest another, that the occurrence of doublets can be related to *small*, stochastic, transient events, as part of the evident state dependency of the phenomenon. The suggestion that doublet occurrence is state dependent is not new (cf. Hoffer *et al.* 1987; Kudina & Alexeeva, 1992), but is made even more attractive by the failure here to find support for alternative explanations. Moreover such a suggestion allows for all of the disparate results in the literature to be reconciled. State dependence can be a catch-all explanation, but it is particularly supported here by some observations of Adams *et al.* (1989), where the three out of twenty-two units of sternocleidomastoid muscle which showed doublets did so only during breathing, but not during head rotation efforts, in which the firing patterns of the units were matched to those during the respiratory efforts. These observations are themselves closely analogous to the present comparisons between the external and internal nerve populations of units, which were well matched for firing frequencies and start times, but which showed a clear difference in doublet occurrence.

Of course it is quite possible to postulate that the intrinsic membrane properties of the two sets of motoneurons here

are sufficiently different to produce the observed incidence of doublets, but there is no independent evidence for this. There is, however, independent evidence from preparations identical to those used here that these two sets of motoneurons receive their synaptic inputs in different ways. This evidence is from Kirkwood & Sears (1989) who found that the synchronization between external intercostal motoneurons in the cat is generally longer in duration than that between either internal intercostal or phrenic motoneurons. Phrenic motoneurons also have not been reported in the cat normally to fire doublets, despite being able to fire reflexly at high frequencies (Nail, Sterling & Widdicombe, 1972), though doublets have been seen in the rabbit during asphyxia (Macefield & Nail, 1987). The interpretation of the longer duration synchronization is that the external nerve motoneurons have more synchronized inputs than the others (Kirkwood & Sears, 1991; Vaughan & Kirkwood, 1993), i.e. the unitary postsynaptic potentials (PSPs) making up their synaptic drive tend, on average, to occur in clusters of a few milliseconds duration.

Such input synchronization is just what could be needed to provide a tendency to re-excite a motoneuron within a few milliseconds of an EPSP which had just fired it. Moreover, motoneuron synchronization is itself known to be highly state dependent, both in normal (Kirkwood *et al.* 1982; Berger, Davies & Sears, 1982) and in pathological situations (Kirkwood, Sears & Westgaard, 1984). Such synchronization can have a wide variety of time courses and strengths (Kirkwood & Sears, 1991), which can represent a variety of mechanisms, but all except the narrowest must represent prolonged or grouped PSPs and variation in these mechanisms could account for many of the different factors that have been reported to influence doublet occurrence. For instance, two of the conditions described by Adams *et al.* (1989) as giving different doublet incidence were also characterized by widely different degrees of motoneuron synchronization, the time course of which most probably involved a large component of input synchronization (Kirkwood & Sears, 1991). Moreover, our hypothesis would also provide an attractive explanation for a remaining discrepancy between the present results and previous ones: when the motoneurons here were placed close to firing threshold by reduction of the CO₂ level, we detected no effect on doublet occurrence, yet for Bawa & Calancie (1983) this procedure was the most potent in promoting them. However, for Bawa & Calancie (1983) this procedure was accompanied by a prolonged period of training to produce the doublets, and it is therefore possible that the subjects did not just place their motoneurons close to threshold, but also were able to change the statistical properties of the synaptic inputs to their motoneurons, just as the subjects of Schmeid, Ivarsson & Fetz (1993) must have done when they produced changes in the level of their motoneuron synchronization with training (cf. Milner-Brown, Stein & Lee, 1975 and discussion in Kirkwood *et al.* 1982).

It should be noted that in this hypothesis it is not the doublets themselves that are represented in the longer durations of the motoneuron synchronization. If one assumes that 50% of external nerve motoneurons fire one doublet on every respiratory cycle (rather more than was observed) and 20 impulses per cycle, then a cross-correlation peak between a pair of such motoneurons would, on average, show an increase in the area of its central peak of only around 4% compared with that which would occur without doublets. However, the observed difference in synchronization among external compared with internal intercostal motoneurons is about an order of magnitude greater than this (Kirkwood & Sears, 1989). What we are proposing is that both differences arise via a common mechanism, a tendency to synchronized inputs leads to doublets in an individual motoneuron and the joint occurrence of the synchronized inputs in pairs of motoneurons produces the relatively long-duration synchronization of the motoneurons.

It should also be noted that we are not claiming that *all* doublets arise through synchronized inputs (a good instance where this would be a rather unlikely hypothesis is the study of Spielman *et al.* (1993) where doublets occurred in motoneurons fired by externally applied currents). Rather we suggest that this is *one* of the factors known to vary in different states or patterns of activation and which would be particularly well suited to be able to promote the firing of any motoneuron in the few milliseconds after a spike, a period which is well known to be one of relative hyperexcitability (Calvin, 1975; Kudina & Churikova, 1990). Our hypothesis of stochastic transient events promoting doublets is distinct, but clearly related to the phenomenon of paired discharges in parkinsonian tremor, which can be related to *periodic* motoneuron synchronization with the frequency of the tremor (4–5 Hz; Elek, Dengler, Konstanzen, Hesse & Wolf, 1991; Baker *et al.* 1992).

A corollary of this hypothesis is that, very like motoneuron synchronization, the occurrence of doublet firing could be regarded as something of an epiphenomenon. Motoneuron synchronization can be functionally important (for instance in ballistic movements), as can doublets, such as in the production of extra tetanic tension (Hoff & Grant, 1944), particularly during fatigue (see Bevan *et al.* 1992 for refs). Circuits and membrane properties exist to enable these functions to be carried out. Perhaps the same circuits and membrane properties are also used (no doubt somewhat reconfigured) for other functions, where elements of the synchronization and/or the doublets are still produced, but with no real physiological advantage, nor any real disadvantage. Moreover, our hypothesis would allow for doublets to be promoted in pathologies, but the occurrence of doublets in itself would not be pathognomic (Kudina & Churikova, 1990; Baker *et al.* 1992; Wiegner, Wierzbicka, Davis & Young, 1993), just as motoneuron synchronization

- can be changed in pathological situations, but not necessarily outside the normal range (Kirkwood *et al.* 1984; Datta, Farmer & Stephens, 1991; Baker *et al.* 1992).
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