

RECRUITMENT OF MOTOR UNITS IN RESPONSE TO TRANSCRANIAL MAGNETIC STIMULATION IN MAN

BY PARVEEN BAWA* AND ROGER N. LEMON

From the Department of Anatomy, Downing Street, Cambridge CB2 3DY

(Received 13 January 1993)

SUMMARY

1. Short-latency responses of single motor units (SMUs) and surface electromyographic activity (EMG) to transcranial magnetic stimulation (TMS) were examined in five different hand and forearm muscles of human subjects.

2. The response probability, P (number of extra spikes in the response peak above background per stimulus), was, in general, higher at the lower voluntary discharge rate of the motor unit than at the higher rate.

3. Increasing the strength of TMS increased the response probability of a tonically firing motor unit and at the same time recruited new units which discharged phasically during the response peak. This demonstrates rate coding and recruitment of motor units by excitatory inputs resulting from TMS when the motoneurone pool is tonically facilitated by a constant voluntary drive.

4. Next, TMS was delivered without any voluntary facilitation of motoneurones. The order of recruitment for up to four different motor units discharged by TMS was compared to that observed with voluntary input. The threshold of recruitment for each of the two inputs was estimated from the surface EMG value at which the unit was recruited. For these motoneurone pools (eleven sets of observations), the order of recruitment was the same with TMS and voluntary inputs.

5. From these data it is concluded that, despite the complex and phasic nature of the descending corticospinal volleys generated by TMS, it produces orderly recruitment and rate coding of motoneurones similar to that found for voluntary activation.

INTRODUCTION

The non-invasive technique of transcranial stimulation has been used to study descending corticospinal inputs to spinal motoneurones in man (see Rothwell, Thompson, Day, Boyd & Marsden, 1991, for a review). Although the technique has been usefully employed in clinical neurophysiology, the stimulus appears to act in a non-specific manner upon the motor cortex in man. It generates activation of many motoneurone pools simultaneously, including agonists and antagonists. Consequently the response of a single motoneurone pool probably results from superposition of many excitatory and inhibitory inputs. Furthermore, the descending

* To whom correspondence should be sent at: Department of Kinesiology, Smith Fraser University, Burnaby, Vancouver, British Columbia, Canada.

corticospinal volley resulting from a single magnetic stimulus consists of multiple waves, probably ensuing from both direct and indirect activation of corticospinal neurones (Day *et al.* 1989; Edgley, Eyre, Lemon & Miller, 1990, 1992). This suggests that the descending input impinging on a motoneurone, resulting from a single transcranial stimulus, is extremely complex. How do motoneurons of the pool respond to this complex input?

It is well known that a tonic excitatory input to the motoneurone pool of a muscle recruits motoneurons in an orderly size-related fashion (see Henneman & Mendell, 1981, for review). As the input to the pool is increased, the already recruited units increase their firing rate while additional units are recruited. These phenomena of rate coding and recruitment during voluntary activation have been demonstrated for various motoneurone pools of the upper limb (Milner-Brown, Stein & Yemm, 1973; Monster & Chan, 1977; Calancie & Bawa, 1985*b*). For phasic inputs, such as peripheral inputs of short duration, rate coding and recruitment have been studied by constructing average response histograms and computing probabilities of responses at various stimulus strengths (Calancie & Bawa, 1985*b*). We have used this technique to examine systematically rate coding and recruitment of motor units in hand and forearm muscles in response to transcranial magnetic stimulation (TMS) in normal human subjects. We have also investigated the effect of firing rate of the motor units on their responses to TMS. This work has been presented before as a short communication (Bawa & Lemon, 1993).

METHODS

Experiments were performed on four healthy adult subjects (three male, one female) ranging in age from 32 to 49 years. Two of the subjects were authors. The hand and forearm muscles (of either side) investigated in this study were first dorsal interosseous (1DI), abductor pollicis brevis (AbPB), flexor digitorum superficialis (FDS), flexor carpi radialis (FCR) and extensor carpi radialis (ECR). These experiments were approved by the local Ethical Committee.

Recording

Surface electromyographic (EMG) activity from the muscle being examined was recorded with a pair of ARBO Ag-AgCl electrodes and a Neurolog preamplifier (NL824; bandpass filter 50 Hz–5 kHz). Single motor unit (SMU) activity was recorded with intramuscular needle electrodes (Calancie & Bawa, 1985*a*). These consisted of a pair of stainless-steel wires (50 μ m diameter) embedded in a 26 gauge needle. The SMU activity was amplified and filtered (50 Hz–10 kHz).

The hand area of the contralateral motor cortex was stimulated with a standard Magstim 200 magnetic stimulator using a 90 mm double-cone coil. The coil was positioned so as to produce short-latency responses in the hand muscles of the relaxed subject with lowest stimulus threshold. Once the optimal position for stimulation was found, the position of the magnetic coil relative to the subject's head was maintained constant by several Velcro straps. The weight of the coil and its lead were counterbalanced by suspending them from an overhead gantry with elastic bands. This arrangement prevented slippage of the coil over the head and minimized discomfort to the subject. Stimuli were applied at a mean rate of between 0.12 and 0.2 s⁻¹. The stimulus trigger, surface EMG and SMU activity were recorded on a Bio-Logic DTR-1800 digital tape-recorder for off-line analysis.

Procedure

A sterilized intramuscular electrode was inserted in the muscle to be studied. For the first set of experiments, the subject was asked to recruit voluntarily a clearly discernible motor unit and, with the help of audio and visual feedback, discharge it at the lowest possible regular discharge rate; this ranged from 6 to 10 impulses s⁻¹. Approximately 100 stimuli of one strength were delivered. While the subject held the same firing rate, the stimulus strength was changed by 1–2% of maximum

stimulator output (1.5 Tesla) and another 100 stimuli delivered. If the subject could maintain steady discharge of an easily discriminable unit for a longer time, two to four different stimulus strengths were tested. Stimulus strengths ranged from 25 to 40% of maximum stimulator output in different subjects. In order to study the effect of SMU firing rate on the response probability, the subject discharged the motor unit initially at a comfortably low rate (< 10 impulses s^{-1}). Approximately 100 stimuli were applied at this rate and then the subject was asked to control the discharge at a distinctly higher rate (> 10 impulses s^{-1}). One hundred stimuli were applied at this higher firing rate. During prolonged recordings from the same unit, the subject rested between delivery of blocks of stimuli.

In the second set of experiments, activation of the muscles with TMS and voluntary drive were done separately. Details are given in the Results.

Analysis

A CED 1401 interface with SIGAVG and Spike2 software were used to analyse the data in the following way:

(i) *Motor unit-triggered average (MU-TA)*. In order to confirm continuous sampling from the same SMU throughout the experiment, the intramuscularly recorded potential (referred to as SMU potential) was discriminated with a double time–amplitude window discriminator. Its output was used to trigger the computer while SMU and unrectified EMG data were digitized on two A–D channels at a rate of 10 kHz; 250–500 sweeps were averaged. The waveform resulting from averaging surface EMG has been referred to as the motor unit-triggered average (MU-TA; Lemon, Mantel & Rea, 1990). The motor unit action potential recorded with an intramuscular needle (2–5 ms in duration) reflects predominantly the activity of a few muscle fibres of the active motor unit and can change in size and shape during long recording periods. The MU-TA on the other hand (10–20 ms in duration) reflects activity of all muscle fibres of the motor unit and remains constant over the period of recording.

(ii) *EMG averaging*. Surface EMG was rectified and averaged (twenty to thirty sweeps) with respect to the stimulus trigger pulses. For these averages EMG was digitized at 1–4 kHz.

(iii) *Peri-stimulus time histograms (PSTHs)*. Spikes of a tonically firing SMU were converted into TTL pulses with the window discriminator. Correction for the delays introduced in generating TTL pulses were made in all latency measurements. SMUs invariably changed in size and/or shape during prolonged activity or during phasic response due to superposition of action potentials from other units. When a motor unit responds twice to a stimulus with a very short interspike interval (ISI), of the order of 4–5 ms, the second spike can be much smaller than the first, and may be missed by the window discriminator. On the other hand, when TMS evokes a very large response, the discriminator may be triggered by action potentials other than that being investigated, resulting in an overestimate of the response probability. In order to minimize such errors, analog SMU data were acquired with a 10 kHz sampling rate, using the CED 1401 interface and Spike2 program, and together with the stimulus and discriminator TTL pulses. For the period 300 ms before and after the stimulus, correct discriminator output was checked for the SMU in question. Incorrect output TTL pulses were deleted while those that had been missed were added. When superimposition of responses from three or more different SMUs prevented correct identification of the SMU being investigated, the corresponding stimulus and the response digital pulses were deleted from the record before constructing PSTHs. If during this sorting the response of a clearly identifiable, non-tonic SMU was seen, a new digital event channel was created for this phasically responding SMU. For the same train of stimuli, PSTHs were constructed for both the tonically firing SMU and the concomitantly recorded phasic motor unit.

To compute the firing rate of the tonically firing SMU, a first-order interval histogram was constructed with a bin width of 1 ms while PSTHs were constructed with a bin width of 0.1 ms. For all PSTHs constructed, the onset and the duration of the response peaks were unequivocally clear. For tonically firing SMUs, the background firing was computed from the period 50 ms before to 10 ms after stimulus onset. After the delivery of n stimuli, the net response probability, P , of the SMU during the peak of duration T ms was calculated as follows:

$$\text{Background count for 60 ms/stimulus} = B \text{ counts}/n.$$

$$\text{Background count for } T \text{ ms, } B_T = (B/60)T.$$

$$\text{Counts in the response peak/stimulus, } P_T = \text{total counts}/n.$$

$$\text{Net response probability, } P = P_T - B_T.$$

The peak onset and duration can vary with the background firing rate and the stimulus strength, but the response probability P is not affected by this variability.

Rate coding

Rate coding and recruitment of motoneurons have been defined for tonic voluntary inputs (Milner-Brown *et al.* 1973). For such inputs both phenomena are essentially deterministic. For a phasic excitatory input, the response of a motoneurone is probabilistic. In this paper we define rate coding in terms of response probability to the phasic volley generated by TMS. Rate coding for a tonic input implies shortening of ISI or an increase in average firing rate. We argue that an increase in response probability also implies shortening of ISI or an increase in the instantaneous firing rate. Consider the model of a tonically firing motoneurone discharging at 10 impulses s^{-1} with an after-hyperpolarization (AHP) amplitude of 10 mV (Ashby & Zilm, 1982). A weak magnetic stimulus generating an excitatory input to the motoneurone equivalent to twenty EPSP units will produce a PSTH peak with a low response probability. A stronger stimulus generating an input equivalent to fifty EPSP units will increase the probability substantially. Inherent in these P values are estimates of shortened ISIs or increased instantaneous firing rates. Thus, in the case of the weak stimulus, some ISIs will shorten from 100 to 80 ms, equivalent to an increase in the instantaneous firing rate from 10 to 12.5 impulses s^{-1} . In the case of the stronger stimulus, the ISI could shorten by up to 50 ms or the instantaneous firing rate would increase to 20 impulses s^{-1} . Thus, rate coding for phasic inputs is indicated by an increased response probability.

RESULTS

Form of the responses to TMS

Data are reported for fifty-five motor units with 104 PSTHs comparing the effects of firing rate of SMUs and stimulus intensity on the probability of response. Recruitment thresholds of most of the units were low, ranging up to approximately 25% of maximum voluntary contraction, as assessed from the surface EMG. Response peaks of SMUs recorded from the forearm muscles ECR, FCR and FDS had the shortest onset latencies, while the peaks of SMUs from hand muscles had longer latencies. For example, in one subject from whom data were available from all five muscles, the onset latencies ranged from 12.0 to 15.7 ms for FCR, 13.3 to 20.7 ms for ECR, 12.7 to 13 ms for FDS, 19.2 to 20.4 ms for 1DI and 20.3 to 23.6 ms for AbPB. The wide range of latencies observed in ECR probably reflected the location of the recording electrode with respect to the motor point of the muscle. Figure 1 shows results from two SMUs recorded from ECR at different times during the same experimental session. For one motor unit (SMU 1; Fig. 1A and B), the onset of the SMU potential coincided with the start of the MU-TA; the onset latency of the PSTH peak was 14.5 ms. For the second unit (SMU 2; Fig. 1C and D), the SMU potential lagged the onset of the MU-TA by 7 ms and the onset latency of the PSTH peak was 21.5 ms. The difference in latency of PSTH peaks (7.0 ms) could thus be accounted for by delay within the muscle.

The duration of response peaks of tonically firing SMUs ranged from 0.8 to 7.2 ms. As reported previously, each response peak, when examined at an expanded time scale, usually consisted of two to three subpeaks (Day *et al.* 1989; Mills, 1991; Palmer & Ashby, 1992). Of the seventy-six PSTHs obtained for thirty-nine tonically firing SMUs, twenty-one PSTHs, from nine units, showed a single peak at all stimulus intensities and at the firing rates we have tested. These single, well-defined peaks ranged in duration from 0.8 to 1.9 ms and were always followed by a period of decreased activity. This post-peak silent period mostly ranged from 30 to 40 ms but was seen to extend to almost 70 ms in a few cases. Nine histograms, from five units,

had two well-defined peaks while ten (from five units) had three. For the remaining histograms, the peaks were broad, but the onset and separation of subpeaks were not well defined. For the phasically responding units, there was a large scatter within each response peak and the subpeaks were not discernible.

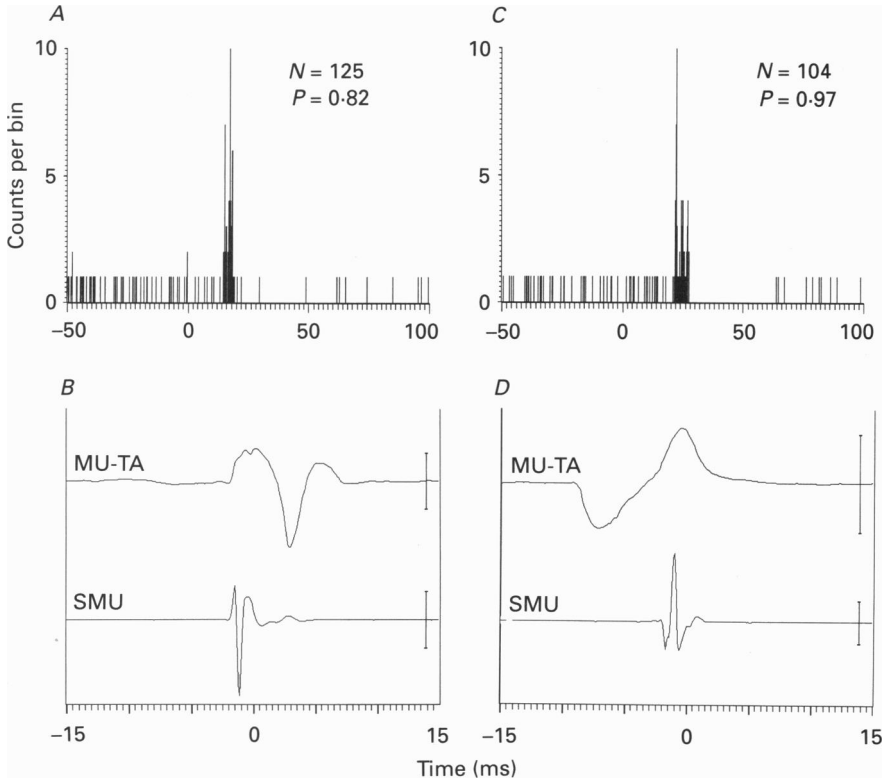


Fig. 1. Peri-stimulus time histograms (PSTH) of two SMUs recorded from ECR muscle with the same intramuscular electrode during the same session but at different times. N , number of TMS stimuli used to compile the PSTH. P , response probability (see text). Onset of the peak of the PSTH is 14.5 ms in *A* and 21.5 ms in *C*. The latency difference of 7 ms in responses from the same muscle may be explained by different delays within the muscle (see text). *B* and *D*, the lower traces (SMU) show the intramuscularly recorded motor unit potentials of the two units. Spike-triggered averages of the unrectified surface EMG with respect to SMU discharges (motor unit-triggered average or MU-TA) are shown above. Time zero marks the TTL output from the window discriminator which was used to trigger the computer for averaging. The onset of MU-TA and the SMU discharge are simultaneous in *B* while the onset of SMU is delayed by 7 ms in *D*. Bin width in *A* and *C*, 100 μ s. Calibration bars in *B* and *D*, 100 μ V. Sampling rate in *B* and *D*, 10 kHz.

In order to compare the magnitudes of the response peaks in our data to those reported previously in the literature we used eqn (1) of Ashby & Zilm (1982) to estimate the magnitude of EPSPs evoked in motoneurons by TMS. For a rhythmically firing motoneurone, if an EPSP falls between two discharges, it can advance the occurrence of the next discharge by an amount which depends upon the amplitude of the EPSP and the phase of the motoneuronal AHP in which it falls. According to the Ashby & Zilm model, for a motoneurone with an AHP of 10 mV

(100 EPSP units) and ISI of 100 ms, an interpolated EPSP of x units brings the motoneurone to firing threshold x ms earlier. Alternatively, for a motoneurone firing at 10 impulses s^{-1} with maximum AHP of 10 mV, ten EPSP units will be equivalent to 1 mV of depolarization. We made estimates of EPSP units for twenty-one PSTHs which showed clear single peaks with the highest TMS intensity used for each of the

TABLE 1. Effect of firing rate on response probability

Unit No.	Stimulus intensity (%)	Firing rate (impulses s^{-1})	Probability P
9 1DI	37	7.14	0.70
		9.52	0.69
		10.00	0.49
10 1DI	33	8.00	0.31
		10.75	0.28
28 FCR	28	< 3	1.01
		11.11	0.42
39 ECR	32	8.83	0.63*
		13.33	0.64
45 ECR	28	10.50	0.57
		12.99	0.40
46 ECR	28	10.87	0.51
		11.77	0.19
48 FCR	39	9.52	0.14
		11.30	0.10
50 FCR	41	8.7	0.12*
		11.5	0.13
51 FCR	33	7.69	0.39
		11.24	0.24
53 1DI	24	8.93	0.48
		10.87	0.39

units. The values ranged between 16.3 and 60.8 EPSP units (mean 42.0 ± 12.4 s.d.). In these cases of a single peak one can assume that the underlying EPSP generated by TMS was simple in form. In addition we estimated EPSP units for all seventy-two PSTHs, including those with responses of several subpeaks, where the interpretation of the underlying EPSP is more complex. The values ranged from 9.1 to 102.3 EPSP units (mean 49.3 ± 24.1). Of these PSTHs with multiple peaks the highest value of EPSP units estimated from only the first peak was seventy-two. Values close to 100 or greater reflect the cases where the motoneurone discharged twice during the PSTH peak in response to a single stimulus. Histograms obtained from units which fired doublets when activated under voluntary control were not included in this analysis because it is not possible to define values of ISIs.

Effects of firing rate on response probability

We tested the effect of TMS on ten SMUs with two to three different firing rates (Table 1). In eight out of ten comparisons, response probability, P , was higher at the lower firing rate of the motoneurone. In the other two cases (* in Table 1), which were

both from the same subject, P was essentially the same at the two rates. Figure 2 illustrates response histograms of a motor unit at two different firing rates. On the left (Fig. 2A and B) are first-order interval histograms to show the two firing rates. The PSTHs (Fig. 2C and D) show that at the lower firing rate of 8.9 impulses s^{-1} , P has a higher value than at the higher firing rate of 10.9 impulses s^{-1} .

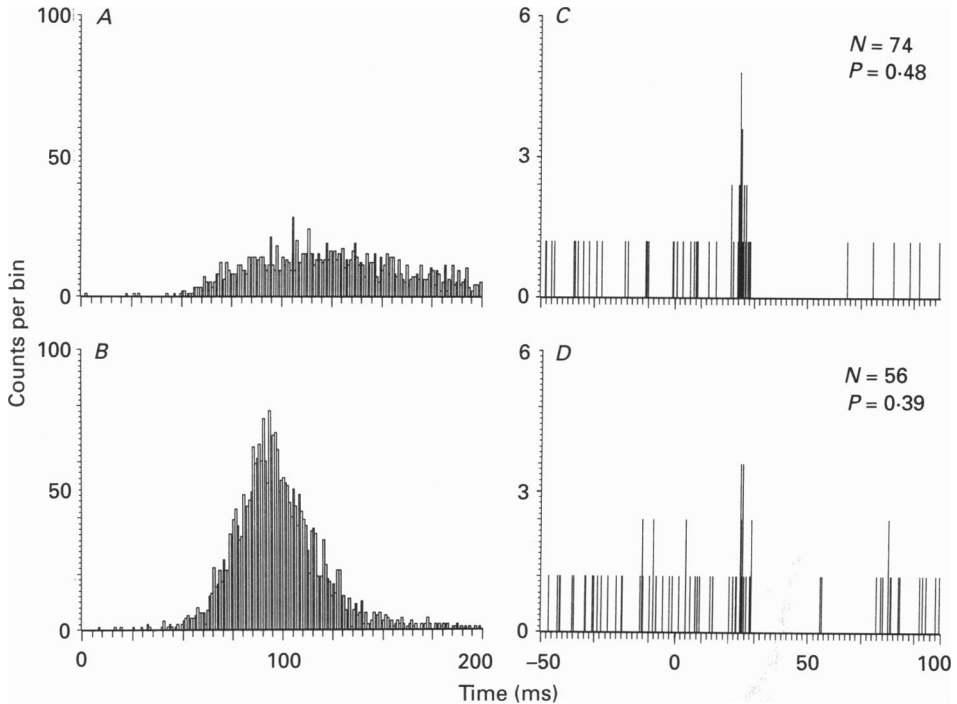


Fig. 2. Effects of firing rate of a 1DI motor unit on its response probability to TMS with an intensity of 24% maximum stimulator output. A and B illustrate first-order interval histograms with a bin width of 1 ms. In A the unit is firing at a rate of 8.9 impulses s^{-1} and in B at 10.9 impulses s^{-1} . Response probability (P) at the lower firing rate (C) is 0.48 and at the higher rate (D) it is 0.39. Bin width for C and D, 100 μs .

Further evidence for the influence of firing rate on responses to TMS came from a comparison of responses in two simultaneously recorded units, with similar voluntary recruitment thresholds. As shown in Table 2, three of the four comparisons (three motor units) studied in this way had quite different firing rates. The response probability of the SMU discharging at a faster rate was always lower than that of the more slowly firing unit.

Rate coding

For twenty SMUs (both tonic and phasic), the response probability was determined at several different stimulus intensities (25–40% of maximum stimulator output), resulting in fifty-seven PSTHs. For each of the twenty units, as the strength of stimulus increased, so did the response probability. For tonically firing SMUs, the response probability, P , ranged from 0.20 to 1.12. Since $P = 1$ means that the unit

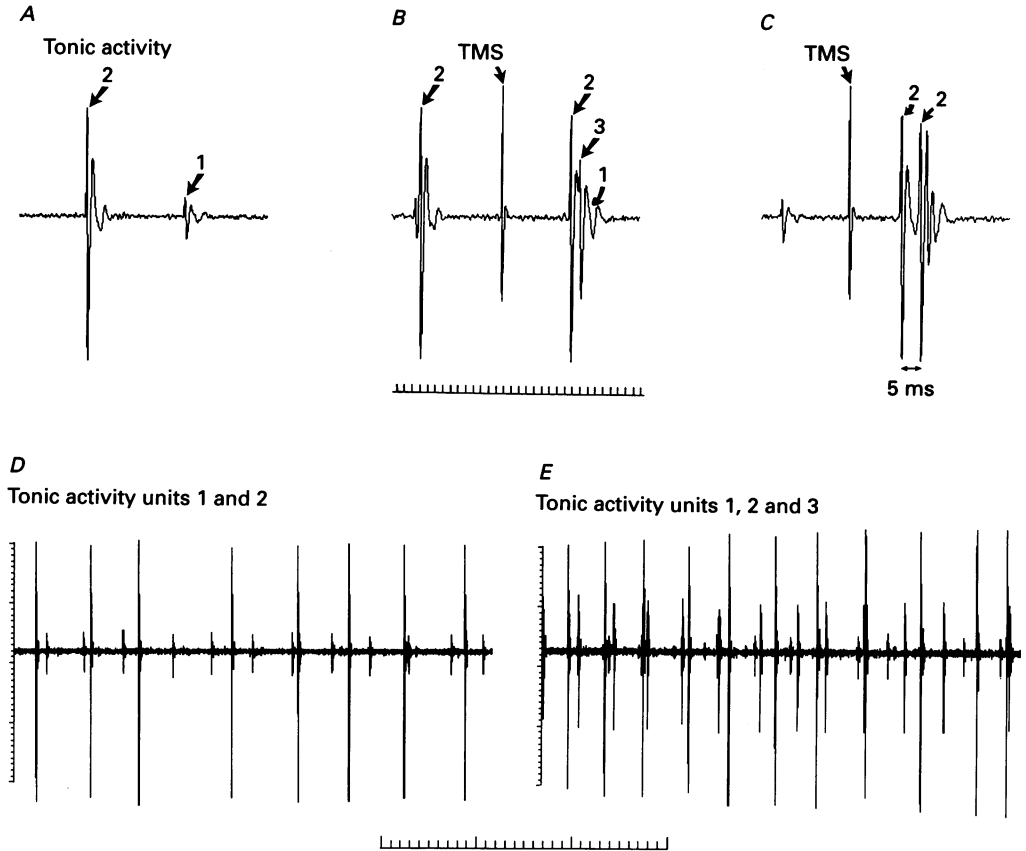


Fig. 3. Raw data for three simultaneously recorded SMUs 1, 2 and 3. Units 1 and 2 were discharged tonically by the subject; the shapes and sizes of the two units, unaffected by TMS, are shown in *A*. *B* illustrates the responses of these two units (1 and 2) and one phasic unit (3) to TMS at 34% intensity. TMS indicated by stimulus artifact. *C* shows that unit 2 responded twice to TMS with an ISI of only 5 ms. *D* illustrates the tonic firing pattern of units 1 and 2, while *E* shows that with a little more voluntary effort, unit 3 could be made to fire tonically. Peak-to-peak amplitude of SMU 2, 0.26 mV. Time markers: top, 2 ms; bottom, 20 ms.

TABLE 2. Comparison of two simultaneously recorded tonic units

Unit No.	Stimulus intensity (%)	Firing rate (impulses s ⁻¹)	Probability <i>P</i>
11 1DI	30	9.71	0.61
12 1DI		12.50	0.31
25 FDS	27	7.41	0.46
24 FDS		8.62	0.42
30 ECR	34	9.50	0.82
32 ECR		12.50	0.46
30 ECR	32	9.30	0.37
32 ECR		11.50	0.26

responded once to every stimulus, a value of $P > 1$ implies that the SMU sometimes discharged twice during the peak in response to a single stimulus. Such short interspike interval (ISI) double discharges were observed in both hand and wrist muscles and in all subjects, especially at higher stimulus intensities. These short ISIs ranged from 4 to 7 ms (Fig. 3C), with both spikes occurring during the response peak. A second type of short ISI occurred when a unit responded once (or even twice) during the peak even though the unit had just been fired voluntarily around the time of application of the stimulus (Fig. 3B). Such short ISI ranged from 10 to 25 ms. One of the units was seen to fire three times within 22 ms.

Figures 3 and 4 illustrate raw data and PSTHs for three units recorded simultaneously from ECR. Units 1 and 2 were voluntarily maintained to fire tonically at constant rates throughout the recording session (Fig. 3A and D) while unit 3 responded only phasically to TMS (Fig. 3B). However, the subject was able to make unit 3 fire tonically by increasing his voluntary drive (Fig. 3E). The PSTHs in Fig. 4 (A–C and D–F) illustrate the phenomenon of rate coding with the phasic volleys generated by TMS. For each of the two tonic units, the higher the stimulus strength, the higher the probability of response. The term ‘rate coding’ is used to describe this phenomenon since a higher response probability implies higher instantaneous firing rates (see Methods). Comparing the two tonically firing units (Fig. 4A–C and D–F), the response probability was greater for the higher threshold unit 2 than for the lower threshold unit 1. This may be explained by the rate dependence of the lower threshold unit firing at a relatively faster rate (see above), and is equivalent to earlier saturation of firing rate of the lower threshold unit seen with tonic excitatory inputs (Monster & Chan, 1977). The lowest P values in this group of units were for the phasic unit (unit 3). This unit responded to only one of 120 shocks delivered at the weakest stimulus intensity (32%). Such comparisons of tonic *versus* phasic units varied from pair to pair. Table 3 shows data for eight pairs for which changes in P with stimulus strength could be compared between tonic and phasic units recorded simultaneously. The difference in P values (between strongest and the weakest stimulus) was much higher for tonic units for three pairs, higher for the phasic units for three pairs and values were comparable for the other two pairs. Rate coding for all tonic and phasic units is illustrated in Fig. 5.

Recruitment of voluntarily activated motor units by TMS

Comparison of Fig. 4C, F and I demonstrates that as the descending volley rate modulated the firing rate of already firing units 1 and 2, it recruited a new unit, unit 3 (Fig. 4I). In other words, TMS produced rate coding and recruitment very similar to that observed with tonic voluntary drive. As the intensity of the phasic input with TMS was increased, the probability of response increased for both the tonically and non-tonically firing units. Without the magnetic stimulus but with extra voluntary effort the subject was able to recruit the phasically responding unit to fire tonically, e.g. unit 3 in Fig. 3E. Thus, the next unit recruited with excitation from the magnetic stimulus was also the next unit recruited tonically by voluntary effort.

Recruitment of additional, phasically responding motor units occurred not only by increasing the intensity of TMS but also by increasing the firing rate of the already tonically firing units, while the stimulus intensity was kept constant. Increasing the

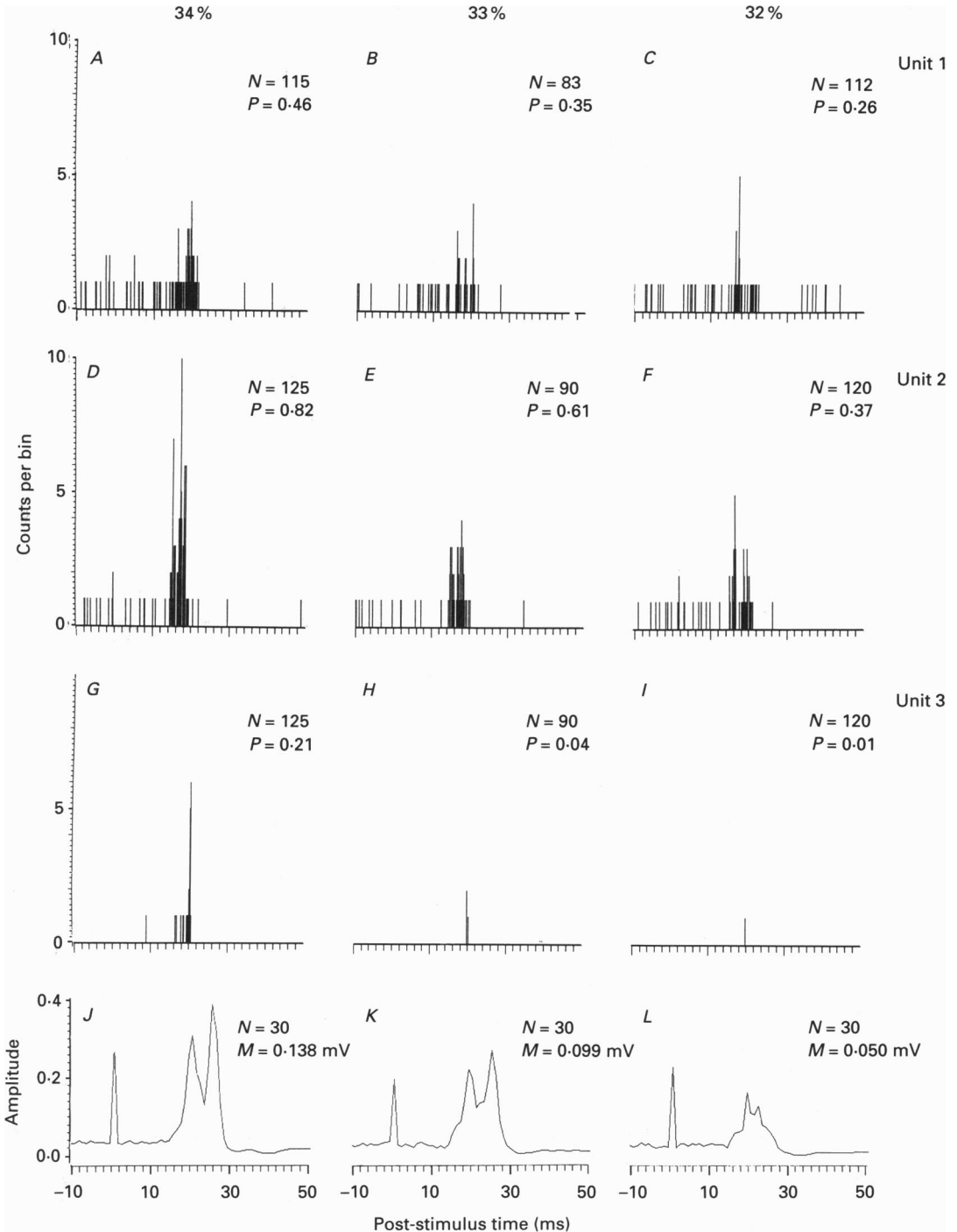


Fig. 4. Rate coding for three simultaneously recorded units from ECR of one subject (same as units 1, 2 and 3 of Fig. 3). The lowest threshold unit (unit 1 of Fig. 3), with responses shown in *A*, *B* and *C*, was voluntarily discharged at 12 impulses s^{-1} . The next higher threshold unit (unit 2 of Fig. 3), with responses shown in *D*, *E* and *F*, was tonically discharged at 9 impulses s^{-1} . The third unit (unit 3 in Fig. 3), with responses shown in *G*,

firing rate of a unit is accompanied by an overall increase in excitability of all motoneurons belonging to the pool of that muscle. Hence, the same intensity of TMS could phasically recruit more units. In other words, the tonic voluntary and the phasic TMS activities complement each other at the motoneuronal level.

Recruitment order of motor units either by voluntary drive or by TMS

A possible criticism of the above procedures and conclusions is that the protocol biased the motoneurone pool with voluntary input (Kernell & Hultborn, 1990;

TABLE 3. Rate coding of simultaneously recorded tonic and phasic units

Unit No.	Stimulus intensity (%)	Tonic unit <i>P</i>	Phasic unit <i>P</i>
3 and 4 ECR	25	0.35	0.00
	28	0.75	0.12
	38	0.84	0.45
13 and 14 AbPB	32	0.34	0.00
	33	0.53	0.10
26 and 27 FDS	26	0.21	0.00
	27	0.29	0.03
	28	0.30	0.12
	30	0.39	0.28
28 and 29 FCR	28	0.31	0.10
	29	0.32	0.68
31 and 32 ECR	32	0.26	0.01
	33	0.35	0.04
	34	0.46	0.21
30 and 31 ECR	32	0.37	0.01
	33	0.61	0.04
	34	0.82	0.21
33 and 34 ECR	32	0.15	0.04
	34	0.42	0.64
39 and 40 ECR	32	0.63	0.01
	33	0.82	0.03
	34	0.87	0.07

Rothwell *et al.* 1991). One can argue that the units recruited phasically with TMS were already facilitated by subthreshold voluntary input. It is not surprising then that an increased input of either type should have recruited the same unit. Therefore, in the next set of experiments, the recruitment order of motor units by the two inputs was examined independently.

The subject was asked to relax completely while SMUs were recruited by TMS alone. With the lowest possible stimulus strength one SMU was recruited. Approximately ten responses of this first unit were recorded, and then the stimulus

H and *I*, responded only phasically to TMS. It can be seen that for each of the three units the response probability, *P*, increased with increasing TMS intensity. The corresponding surface EMG responses at the three TMS intensities are shown in *J*, *K* and *L*. *M* is the average response amplitude in millivolts during the peak above background. Bin width for PSTHs, 100 μ s; sampling rate for surface EMG, 1 kHz.

was increased to recruit a second, clearly discernible unit. Again, approximately ten responses of the second unit (with the first unit, but without any additional units) were recorded and then TMS intensity was increased to record the next clear unit. Two to four clearly discriminable units were obtained in each set. Next, TMS was

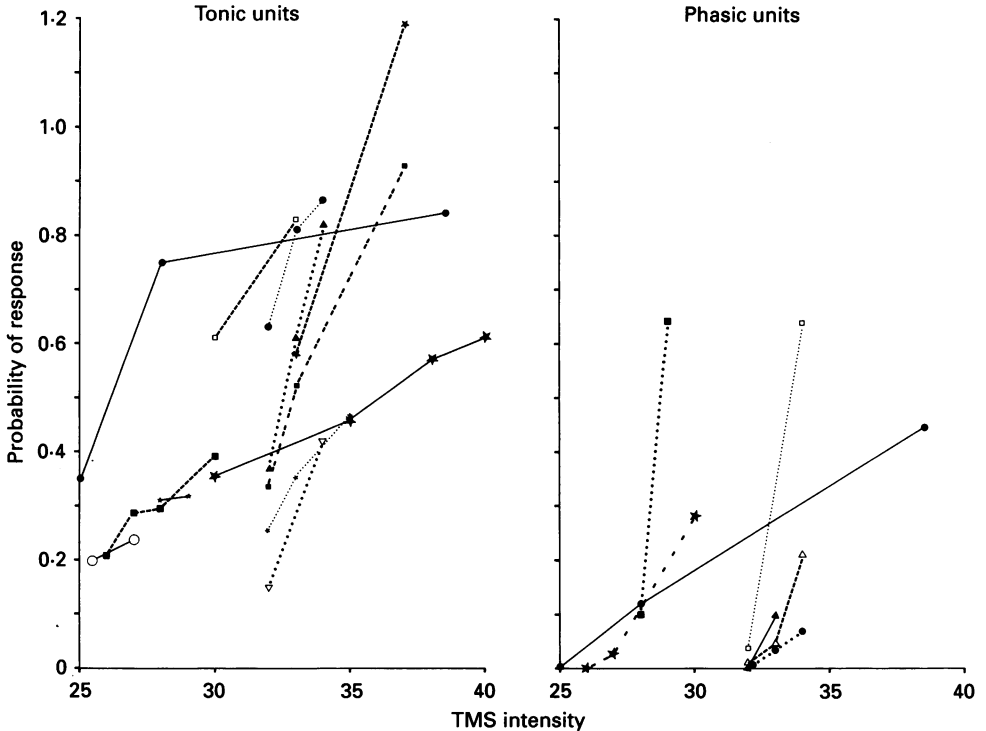


Fig. 5. Changes in response probability of SMUs with increasing TMS intensity. Left graph shows the behaviour of thirteen units which were discharging tonically when TMS was applied. The graph on the right shows the behaviour of seven units which responded to TMS alone (the subject did not fire them voluntarily).

stopped and the subject was asked to recruit the same units voluntarily. After one of the units identified with TMS was recruited voluntarily, the subject held that unit for 30 s before the next identified unit was recruited. The left-hand panels of Fig. 6 illustrate data from a set of three motor units (SMU 1, 2, 3). At the lowest stimulus strength used (top panel), at which SMU 1 was recruited, the amplitude of the simultaneously recorded surface EMG response (rectified) is shown in the upper trace. The mean value of approximately ten such rectified responses was computed to obtain an estimate of the recruitment threshold of SMU 1. The amplitude of the surface EMG response was also determined for the higher intensities used to recruit SMU 2 and 3 (middle and lower panels, respectively). This method of obtaining estimates of recruitment thresholds by TMS is similar to rank ordering of motoneurons from population reflex responses recorded in the ventral roots in animal studies (Clamann, Gillies, Skinner & Henneman, 1974). The right-hand panels in Fig. 6 illustrate the levels of voluntary surface EMG recorded when SMUs 1, 2 and

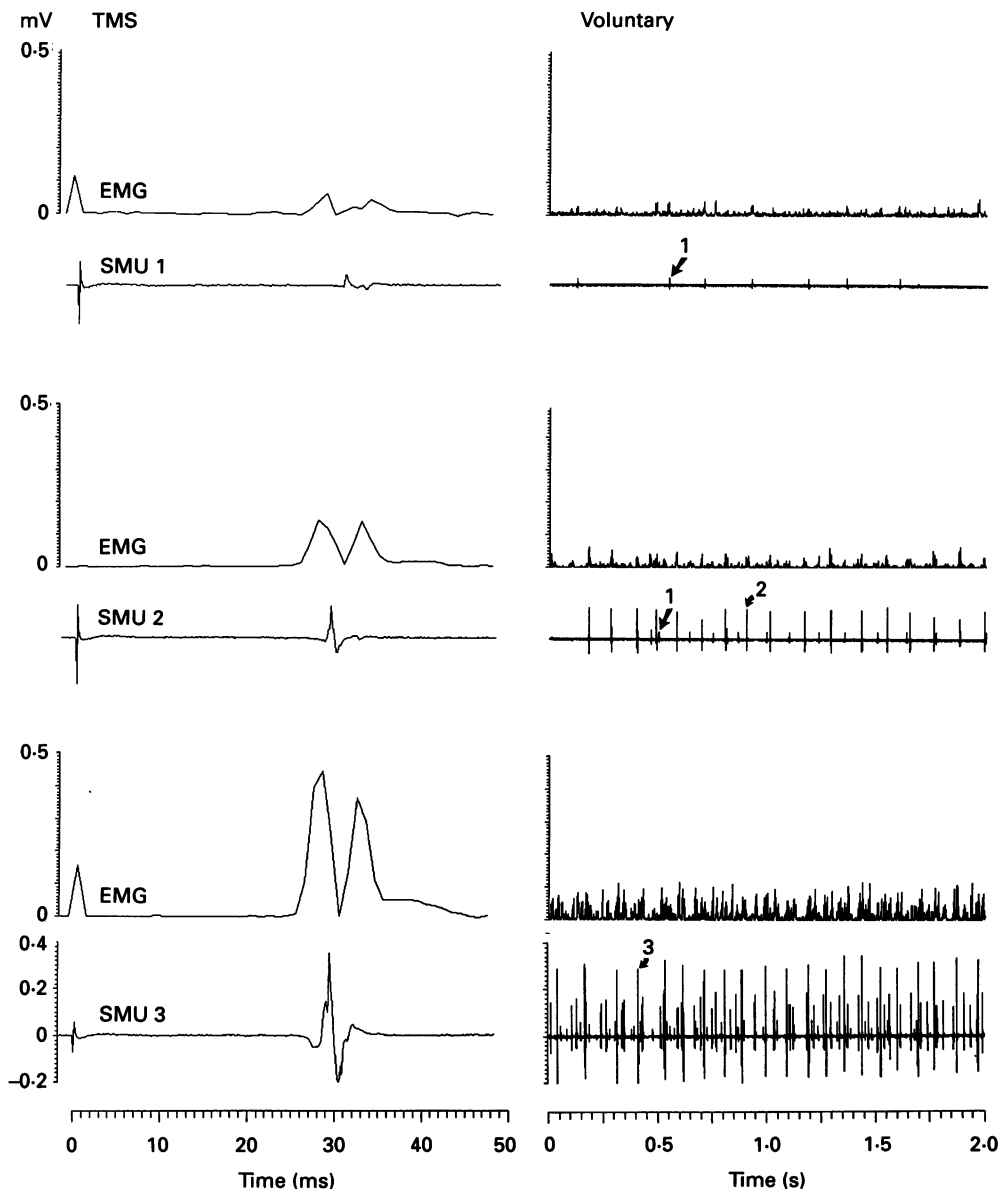


Fig. 6. Obtaining the recruitment thresholds for motor units separately for TMS and for voluntary excitation. Single motor unit data were digitized at 10 kHz and EMG data at 1 kHz; the latter were then rectified. With TMS, the representative surface EMG responses at which units 1, 2 and 3 were recruited consistently are shown in the left three panels. Recruitment threshold for a unit was estimated from the mean amplitude of the EMG average response (average of ten responses). Levels of voluntary EMG at which units 1, 2 and 3 were recruited tonically are shown on the right. The average level of surface EMG over a 2 s period when the unit fired tonically was used as an estimate of voluntary recruitment threshold.

3 discharged tonically. In this study, the amplitude of the surface EMG (averaged over 2 s) at which a unit started to fire tonically was taken as its recruitment threshold. For each set of units studied in any one session, recruitment thresholds with TMS were plotted against recruitment threshold values obtained with voluntary

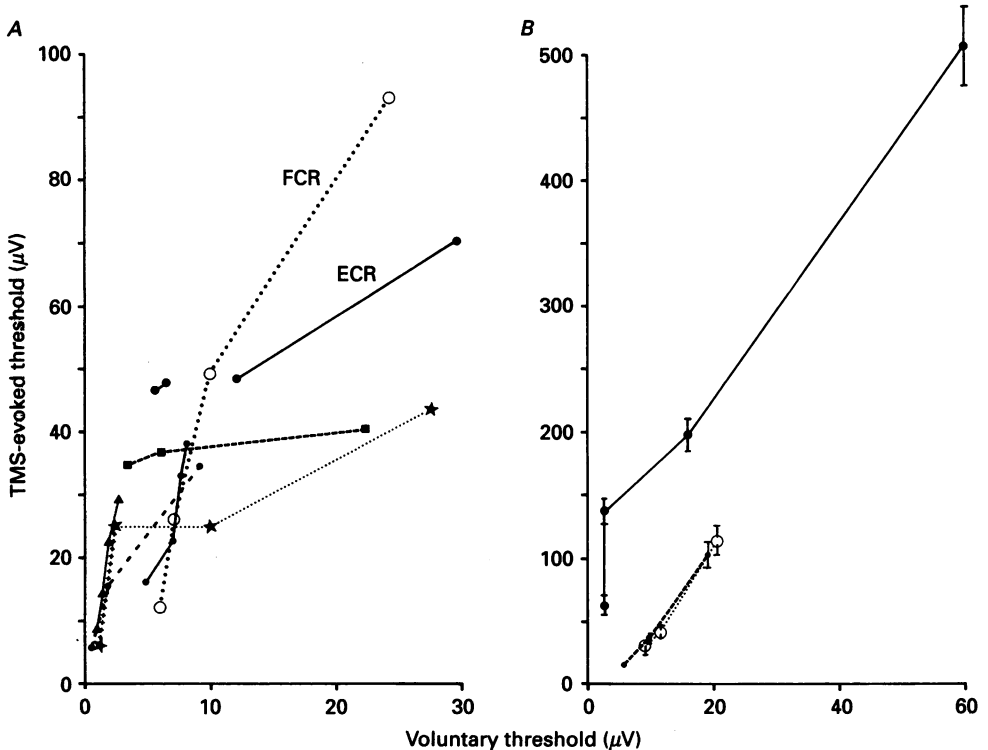


Fig. 7. Correlations of voluntary *versus* phasic response thresholds of SMUs measured in terms of the amplitude of surface EMG when the unit was recruited by either TMS (ordinate) or by voluntary activation (abscissa). All data were recorded from 1DI except the two sets labelled FCR and ECR in *A*. Eight sets are shown in *A*. Only three sets are shown in *B* in order to illustrate values of standard error (s.e.m.) bars for the data obtained from two subjects.

activation. Figure 7 shows results from eleven sets of SMUs. The eight sets plotted on the left showed a clear positive correlation between the two different estimates of recruitment thresholds. Discharge of a motoneurone to a phasic excitatory input, particularly under resting conditions, is probabilistic in nature. As a result, the magnitudes of surface EMG response to the same TMS intensity were quite variable. This variability is illustrated by standard error (s.e.m.) bars on three sets of data plotted on the right of Fig. 7. The sizes of the s.e.m. bars are small confirming that, for most of the data sets, the recruitment thresholds of motor units within one set were well separated.

In this protocol rate coding was not assessed quantitatively because the high stimulus intensities used were uncomfortable for the subjects and generally made it difficult to record for long periods from any one motor unit.

In addition to the well-identified units in a set, there were units recruited with TMS which could not be identified during voluntary activation and *vice versa*. This can be seen in the lower right panel of Fig. 7. There were more than three units active but only three were identified with both TMS and voluntary activation.

DISCUSSION

Recruitment of motor units

Orderly recruitment of motoneurons in human limb muscles has been well documented for reflex and voluntary excitatory inputs to the motoneurone pool (see Calancie & Bawa, 1990, for a review). A similar pattern of recruitment generated by descending inputs excited by transcranial electrical or magnetic cortical stimulation has been suggested by Hess & Mills (1986) and Gandevia & Rothwell (1987). These authors showed that the motor unit first recruited by voluntary contraction was also the first to respond to transcranial cortical stimulation. Both studies tested only the first recruited unit. The finding that the lowest threshold unit for voluntary input was also the first to respond to cortical stimulation is in contrast to some earlier reports on descending inputs. Thus activation of corticospinal input to extensor digitorum motoneurons in the baboon (Clough, Kernell & Phillips, 1968) and/or of rubrospinal input to cat triceps surae motoneurons (Burke, Jankowska & ten Bruggencate, 1970) was found to produce the largest EPSPs in the larger motoneurons, which, according to the size principle, would be recruited at higher thresholds than smaller motoneurons. Milner-Brown, Girvin & Brown (1975), in their study of electrical stimulation of the scalp in human patients, also suggested preferential recruitment of larger motoneurons. Conversely, recruitment order from smaller to larger motoneurons with increasing intensities of scalp stimulation was deduced by Calancie, Nordin, Wallin & Hagbarth (1987) since the onset of the surface EMG response was earlier with stronger stimuli, suggesting recruitment of faster, and hence larger, motoneurons with stronger stimuli. The present study has supported these initial observations of Calancie *et al.* (1987) by examining in detail the sequential recruitment order of motoneurons.

It has been demonstrated that magnetic stimulation of the motor cortex produces rate coding and recruitment of forearm and hand muscle motoneurons similar to that reported for voluntary inputs. Rate coding, or an increase in firing probability with increasing intensity of TMS, has been shown previously for FCR (Calancie *et al.* 1987), deltoid (Colebatch, Rothwell, Day, Thompson & Marsden, 1990) and 1DI motor units (Boniface, Mills & Schubert, 1991). The present study has demonstrated that as rate coding of tonically firing units proceeds with increasing stimulus intensity, the magnetic stimulus recruits new units to respond phasically. This pattern is very similar to that observed by Monster & Chan (1977) for tonic voluntary inputs. This protocol, of course, biases the responses to magnetic stimulation with voluntary inputs. However, by separating the two inputs we have clearly demonstrated that the order of recruitment for magnetic stimulation is the same as for voluntary input.

The relationship between voluntary recruitment thresholds and TMS-evoked thresholds varied for different sets of motor units, as shown by differences in the

slopes in Fig. 7. Several factors may contribute to these differences. During TMS, responses recorded from FCR and ECR would certainly be contaminated by responses originating in other forearm muscles, also activated by TMS. However, during voluntary contraction, the surface electrodes would have recorded EMG from the active wrist flexors and extensors only. The contamination of EMG may be less problematic for 1DI. But apart from these technical difficulties, the different slopes could also indicate the quite different susceptibilities of different motor units to TMS. This is supported by the wide range of response probabilities obtained from different motor units in the first experiment. It is known from animal experiments that there is a considerable range in the amplitude of the compound monosynaptic EPSP elicited in hand and forearm motoneurons by a supramaximal stimulus to the pyramidal tract (see Lemon, 1990).

In the second set of experiments, there were some motor units which were identifiable only with one type of input. Some SMU potentials may have changed shape and size between the two recording situations; an SMU potential could look quite different when recorded with the muscle at rest (with TMS) and when the muscle was actively contracted with voluntary input. A second possibility is that the two inputs recruit separate subpopulations of a motoneurone pool with some overlapping units. It is this overlapping subpopulation that we were able to identify with both inputs. There is, however, no evidence of subpopulations in the muscles we have studied (Desmedt & Godaux, 1981; Riek & Bawa, 1992).

Motor unit responses to single TMS

In response to a single stimulus, a motor unit, tonic or non-tonic, usually responded with a single spike during the response peak of the PSTH, and we have argued above that the advance in the timing of motor unit discharge that this represents is equivalent to rate coding seen with other inputs. However, double firing to a single stimulus was observed in several units. In motor units which discharged doublets during their tonic firing under voluntary control, very short interspike intervals (5 ms) were also seen in response to TMS, even at low intensities. These doublets may have resulted from delayed depolarization in some motoneurons (Bawa & Calancie, 1983). On the other hand, double firings seen in other units at higher stimulus intensities were probably due to a large and complex EPSP generated by multiple volleys in the corticospinal tract (Kernell & Wu, 1967; Gandevia & Rothwell, 1987; Day *et al.* 1987, 1989). These double firings, whether resulting from delayed depolarization and/or repetitive corticospinal volleys, could result in a response probability $P > 1$, as we have observed.

Multiple peaks in averaged responses to TMS

When a motor unit responds with a single discharge to a single stimulus, this discharge can occur at two or even three preferred latencies, giving rise to multiple peaks in the averaged response, i.e. in the PSTH (Day *et al.* 1989; Boniface *et al.* 1991; Palmer & Ashby, 1992). The most convincing explanation of these multiple peaks is that they reflect the multiple corticospinal volleys produced by TMS (Day *et al.* 1989; Mills, 1991). These volleys have been recorded directly in monkeys (Edgley *et al.* 1990), and in human subjects (Burke, Hicks, Stephen, Gandevia, Woodforth & Crawford,

1993), and both studies suggest that the earliest volley results from direct excitation of the corticospinal neurones at the level of the cortex (D wave), with subsequent volleys arising from indirect activation of the same neurones (I waves). The relative amplitude of the different subpeaks depends on the strength of TMS and the state of the motoneurone receiving the volley (Mills, 1991). If the state of the motoneurone is such that the first descending volley cannot discharge it, the second volley may produce a large enough EPSP, which by temporal summation with the first one may discharge the motoneurone (Kernell & Wu, 1967). Thus instead of the first peak, the second will dominate. But there is evidence to suggest that the pattern of response is not determined simply by the timing and amplitude of the corticospinal volleys. Multiple peaks are not seen in all motoneurones; in Palmer & Ashby's (1992) study of different upper limb muscles four-fifths showed single peaks. One-third of responsive 1DI units reported by Boniface *et al.* (1991) showed them, and a similarly small (9/39 motor units) proportion was found in the present study. Units with either single or multiple peaks showed these distinctive properties at all intensities used, and this indicates that a single peak is not necessarily due to the use of a weak stimulus (cf. Day *et al.* 1989). Furthermore, in cases of units with multiple peaks, although the first peak was generally the dominant one, this was not true for all units. The form of the peak did not appear to be altered by the different firing rates tested in our study. Thus spinal mechanisms and motoneurone properties may have a dominant influence on the presence and relative magnitudes of these subpeaks. Such an influence is suggested by the observation that the same block of stimuli elicited multiple peaks in an FCR motor unit (total duration, 6.9 ms) and a single peak of 1.1 ms duration in an ECR unit. The units were recorded simultaneously and both fired tonically at about the same rate while the subject performed an isometric radial deviation of the wrist.

Amplitudes of PSTH peaks

A simple large EPSP produces a sharp, well-defined PSTH peak while a complex EPSP produces a broader PSTH peak (Fetz & Gustafsson, 1983). If one assumes that the first subpeak occurs in response to a single, synchronous descending volley produced by direct activation of the cortico-motoneuronal system by TMS (Edgley *et al.* 1990, 1992), then computation of EPSP size from the first subpeak would indicate the strength of the monosynaptic cortico-motoneuronal connection. Using the equation of Ashby & Zilm (1982), Day *et al.* (1987) estimated the largest EPSP value at 3 mV (thirty EPSP units) for 1DI motoneurones excited by transcranial electrical stimulation. Later, Day *et al.* (1989) revised this maximum value to 5 mV (fifty EPSP units) using the same stimulus. They suggested that 5 mV was the absolute maximum since any further increase in intensity saturated the response at $P = 0.5$. With TMS, even lower mean values of 2.9 mV have been quoted for 1DI motor units by Palmer & Ashby (1992), although these authors used only moderate stimulus intensities.

The maximum response probabilities to TMS found that we report are substantially higher than found in the studies referred to above, although we also did not use maximal intensities. For the first subpeak only, the maximum value was seventy-two EPSP units for an ECR motoneurone when it was firing at slightly less

than 10 impulses s^{-1} . There are several factors which could have contributed to these high values. First, technical difficulties may prevent assessment of the maximum EPSP values in motoneurons of distal limb muscles. If one were to assume that at lower force levels recruitment is more dominant in the distal limb motoneurons and rate coding is more dominant in more proximal motoneurons (Kukulka & Clamann, 1981), then it is possible that one can use much higher stimulus intensities to study wrist musculature than 1DI without interference from superposition of additional motor units. Motor units in the hand muscles would be expected to receive larger compound EPSPs from the corticospinal tract than more proximal muscles (Clough *et al.* 1968; Lemon, 1990; Palmer & Ashby, 1992). Nevertheless, the powerful recruitment of additional units in the distal muscles by strong stimuli precludes measurement of responses in a given motor unit when stimuli intense enough to activate the entire cortico-motoneuronal colony projecting to that unit are employed. This problem is less important for more proximal muscles, and may explain why it is possible to record high response probabilities in deltoid (Colebatch *et al.* 1990) and why, in the present study, the largest effects were seen in motor units recorded in wrist rather than in intrinsic hand muscles.

A second factor that may have enhanced the probability of response to TMS is the use of low motor unit firing rates. In 1989, Brouwer, Ashby & Midroni showed that tibialis anterior motor units responded to TMS with a higher probability at slow compared to fast firing rates. Our data for upper limb motoneurons support these observations. When the same unit was examined at two different firing rates, the response probability was higher at the slower rate for eight out of ten units. Furthermore, data from simultaneously recorded units showed that the unit firing at the slower rate responded with a higher probability. For forty of the PSTHs compiled for tonically firing units, the firing rates were less than 9.5 impulses s^{-1} . Finally, because we subjected each motor unit response to critical scrutiny, some responses obtained at higher intensities, which, because of some contamination with other units, would not have been detected by the discriminator, were included in our final PSTHs, resulting in larger response peaks.

The full impact of the cortical stimulus on a motor unit's discharge can be revealed by considering response probabilities of the whole PSTH peak rather than only the first subpeak. *P* values close to unity calculated for all spikes in the peak indicate that the EPSP is large enough to make the motoneurone discharge twice at instantaneous rates close to 200 impulses s^{-1} which corresponds to the secondary firing range of the motoneurone (Kernell, 1965). It should be remembered that the later excitatory subpeaks may be subjected to inhibition (Davey, Romaguère, Maskill & Ellaway, 1992; Ferbert, Priori, Rothwell, Day, Colebatch & Marsden, 1992).

Conclusions

Two important conclusions can be drawn from this work. The first concerns the role of motoneurone properties in determining the order of recruitment. Since the complex and non-specific corticospinal volley generated by TMS can recruit motor units in an orderly fashion, then this suggests that intrinsic motoneuronal properties must play a major role in determining the order of recruitment. Secondly, the high probability of responses in motor unit PSTHs demonstrates the strong impact of

each cortical stimulus on spinal motoneurons. Such strong responses probably result from the combination of direct activation of the cortico-motoneurone pathway by the magnetic stimulus and the density of synapses contributed by this pathway onto hand and forearm motoneurone pools.

The authors are grateful to Mrs Rosalyn Cummings for her help with the figures. This work was supported by Action Research, the MRC and the East Anglian Regional Health Authority and by the Natural Sciences and Engineering Research Council of Canada. P. Bawa was on sabbatical leave from Simon Fraser University, Vancouver, Canada.

REFERENCES

- ASHBY, P. & ZILM, D. (1982). Relationship between EPSP shape and cross-correlation profile explored by computer simulation for studies on human motoneurons. *Experimental Brain Research* **47**, 33–40.
- BAWA, P. & CALANCIE, B. (1983). Repetitive doublets in human flexor carpi radialis muscle. *Journal of Physiology* **339**, 123–132.
- BAWA, P. & LEMON, R. N. (1993). Recruitment of motoneurons by transcranial magnetic stimulation in man. *Journal of Physiology* **467**, 99P.
- BONIFACE, S. J., MILLS, K. R. & SCHUBERT, M. (1991). Responses of single spinal motoneurons to magnetic brain stimulation in healthy subjects and patients with multiple sclerosis. *Brain* **114**, 643–662.
- BROUWER, B., ASHBY, P. & MIDRONI, G. (1989). Excitability of corticospinal neurons during tonic muscle contractions in man. *Experimental Brain Research* **74**, 649–652.
- BURKE, D., HICKS, R., GANDEVIA, S. C., STEPHEN, J., WOODFORTH, I. & CRAWFORD, M. (1993). Direct comparison of corticospinal volleys in human subjects to transcranial magnetic and electrical stimulation. *Journal of Physiology* **470**, 383–393.
- BURKE, R. E., JANKOWSKA, E. & TEN BRUGGENCATE, G. (1970). A comparison of peripheral and rubrospinal synaptic input to slow and fast twitch motor units of triceps surae. *Journal of Physiology* **207**, 709–732.
- CALANCIE, B. & BAWA, P. (1985a). Firing patterns of human flexor carpi radialis motor units during the stretch reflex. *Journal of Neurophysiology* **53**, 1179–1193.
- CALANCIE, B. & BAWA, P. (1985b). Voluntary and reflexive recruitment of flexor carpi radialis motor units in humans. *Journal of Neurophysiology* **53**, 1194–1200.
- CALANCIE, B. & BAWA, P. (1990). Motor unit recruitment in humans. In *The Segmental Control*, ed. BINDER, M. D. & MENDELL, L. M., pp. 75–95. Oxford University Press, New York.
- CALANCIE, B., NORDIN, M., WALLIN, U. & HAGBARTH, K.-E. (1987). Motor-unit responses in human wrist flexor and extensor muscles to transcranial cortical stimulation. *Journal of Neurophysiology* **58**, 1168–1185.
- CLAMANN, H. P., GILLIES, J. D., SKINNER, R. D. & HENNEMAN, E. (1974). Quantitative measures of output of a motoneuron pool during monosynaptic reflexes. *Journal of Neurophysiology* **37**, 1328–1337.
- CLOUGH, J. F. M., KERNELL, D. & PHILLIPS, C. G. (1968). The distribution of monosynaptic excitation from the pyramidal tract and from primary spindle afferents to motoneurons of the baboon's hand and forearm. *Journal of Physiology* **198**, 145–166.
- COLEBATCH, J. G., ROTHWELL, R. C., DAY, B. L., THOMPSON, P. D. & MARSDEN, C. D. (1990). Cortical outflow to proximal arm muscles in man. *Brain* **113**, 1843–1856.
- DAVEY, N. J., ROMAIGUÈRE, P., MASKILL, D. W. & ELLAWAY, P. H. (1992). Is the inhibition of voluntary contraction evoked by transcranial magnetic stimulation in man occurring at the level of the cerebral cortex? *Journal of Physiology* **459**, 150P.
- DAY, B. L., DRESSLER, D., MAERTENS DE NOORDHOUT, A., MARSDEN, C. D., NAKASHIMA, K., ROTHWELL, J. C. & THOMPSON, P. D. (1989). Electric and magnetic stimulation of human motor cortex: surface EMG and single motor unit responses. *Journal of Physiology* **412**, 449–473.
- DAY, B. L., ROTHWELL, J. C., THOMPSON, P. D., DICK, P. R., COWAN, J. M. A., BERARDELLI, A. & MARSDEN, C. D. (1987). Motor cortex stimulation in intact man. 2. Multiple descending volleys. *Brain* **110**, 1191–1209.

- DESMEDT, J. E. & GODAUX, E. (1981). Spinal motoneuron recruitment in man: deordering with direction, but not with speed of voluntary movement. *Science* **214**, 933–936.
- EDGLEY, S. A., EYRE, J. A., LEMON, R. N. & MILLER, S. (1990). Excitation of the corticospinal tract by electromagnetic and electrical stimulation of the scalp in the macaque monkey. *Journal of Physiology* **425**, 301–320.
- EDGLEY, S. A., EYRE, J. A., LEMON, R. N. & MILLER, S. J. (1992). Direct and indirect activation of corticospinal neurones by electrical and magnetic stimulation in the anaesthetized macaque monkey. *Journal of Physiology* **446**, 224P.
- FERBERT, A., PRIORI, A., ROTHWELL, J. C., DAY, B. L., COLEBATCH, J. G. & MARSDEN, C. D. (1992). Interhemispheric inhibition of the human motor cortex. *Journal of Physiology* **453**, 525–546.
- FETZ, E. E. & GUSTAFSSON, B. (1983). Relation between shapes of post-synaptic potentials and changes in firing probability of cat motoneurons. *Journal of Physiology* **341**, 387–410.
- GANDEVIA, S. C. & ROTHWELL, J. C. (1987). Knowledge of motor commands and the recruitment of human motoneurons. *Brain* **110**, 1117–1130.
- HENNEMAN, E. & MENDELL, L. M. (1981). Functional organization of motoneuron pool and its inputs. In *Handbook of Physiology*, section 1, *The Nervous System*, vol. II, *Motor Control*, part 1, ed. BROOKHART, J. M. & MOUNTCASTLE, V. B., pp. 423–507. American Physiological Society, Bethesda, MD, USA.
- HESS, C. W. & MILLS, K. R. (1986). Low-threshold motor units in human hand muscles can be selectively activated by magnetic stimulation. *Journal of Physiology* **380**, 62P.
- KERNELL, D. (1965). The adaptation and the relation between discharge frequency and current strength of cat lumbosacral motoneurons stimulated by long-lasting injected currents. *Acta Physiologica Scandinavica* **65**, 65–73.
- KERNELL, D. & HULTBORN, H. (1990). Synaptic effects on recruitment gain: a mechanism of importance for the input–output relations of motoneurone pools? *Brain Research* **507**, 176–179.
- KERNELL, D. & WU, C.-P. (1967). Post-synaptic effects of cortical stimulation on forelimb motoneurons in the baboon. *Journal of Physiology* **191**, 673–690.
- KUKULKA, C. G. & CLAMANN, H. P. (1981). Comparison of the recruitment and discharge properties of motor units in human brachial biceps and adductor pollicis during isometric contractions. *Brain Research* **219**, 45–55.
- LEMON, R. N. (1990). Mapping the output functions of the motor cortex. In *Signal and Sense: Local and Global Order in Perceptual Maps*, ed. EDELMAN, G., GALL, E. & COWAN, W. M., pp. 315–356. Wiley, Chichester.
- LEMON, R. N., MANTEL, G. W. H. & REA, P. A. (1990). Recording and identification of single motor units in the free-to-move primate hand. *Experimental Brain Research* **81**, 95–106.
- MILLS, K. R. (1991). Magnetic brain stimulation: a tool to explore the action of the motor cortex on single human spinal motoneurons. *Trends in Neurosciences* **14**, 401–405.
- MILNER-BROWN, H. S., GIRVIN, J. P. & BROWN, W. F. (1975). The effects of motor cortical stimulation on the excitability of spinal motoneurons in man. *Canadian Journal of Neurological Sciences* **2**, 245–253.
- MILNER-BROWN, H. S., STEIN, R. B. & YEMM, R. (1973). The orderly recruitment of human motor units during voluntary isometric contractions. *Journal of Physiology* **230**, 359–370.
- MONSTER, A. W. & CHAN, H. (1977). Isometric force production by motor units of extensor communis muscle in man. *Journal of Neurophysiology* **40**, 1432–1443.
- PALMER, E. & ASHBY, P. (1992). Corticospinal projections to upper limb motoneurons in humans. *Journal of Physiology* **448**, 397–412.
- RIEK, S. & BAWA, P. (1992). Recruitment of motor units in human forearm extensors. *Journal of Neurophysiology* **68**, 100–108.
- ROTHWELL, J. C., THOMPSON, P. D., DAY, B. L., BOYD, S. & MARSDEN, C. D. (1991). Stimulation of the human motor cortex through the scalp. *Experimental Physiology* **76**, 159–200.