

Excitability of human upper limb motoneurons during rhythmic discharge tested with transcranial magnetic stimulation

E. Olivier*, P. Bawa †† and R. N. Lemon*

*Department of Anatomy, University of Cambridge, Cambridge, UK
and †Simon Fraser University, School of Kinesiology, Burnaby, British Columbia,
Canada V5A 1S6

1. The activity of thirty-one single motor units (SMUs) was recorded from forearm and hand muscles of three volunteers. The excitability of the rhythmically firing motoneurons supplying these SMUs was examined after voluntary discharge using transcranial magnetic stimulation (TMS).
2. The magnetic stimulus was delivered either at a fixed delay (range: 1–60 ms) after SMU discharge (triggered mode) or at random with respect to voluntary SMU discharge (random mode). Post-stimulus time histograms (PSTHs) of responses to 50–100 stimuli were constructed for each experimental condition.
3. In the *triggered mode*, the probability of response to TMS progressively decreased as the spike-to-stimulus interval was shortened. Shortening of the interval also resulted in redistribution of responses within the different subpeaks characterizing the short-latency response of motor units to TMS: the relative response probability of the first subpeak decreased with the shorter spike-to-stimulus intervals.
4. In the *triggered mode*, the probability of SMU responding to TMS was significantly higher when the firing rate of the motor unit was increased from a slow rate (<10 impulses s^{-1}) to a faster rate (>12 impulses s^{-1}), irrespective of the spike-to-stimulus interval. In contrast, in the *random mode*, the response probability was greater at the slower discharge rate.
5. The higher excitability of motoneurons at the fast rate in the triggered mode is consistent with findings in cat motoneurons suggesting a shallower after-hyperpolarization, but other mechanisms could contribute. Furthermore, our results suggest that there is an asymptotic recovery in the excitability of slow firing motoneurons towards the end of the interspike interval.

Transcranial magnetic stimulation (TMS) of the human motor cortex excites the corticospinal system and produces a short-latency excitation of spinal motoneurons (Barker, Jalinous & Freeston, 1985; Hess, Mills & Murray, 1987; for review see Rothwell, Thompson, Day, Boyd & Marsden, 1991). In this study we have made a detailed investigation of the excitability of human motor units, using the corticospinal volley generated by TMS as a convenient probe.

We have looked at three interrelated features of motoneuronal excitability. Firstly, we have examined changes in response probability obtained with stimuli delivered at fixed intervals after voluntary discharge of the unit. Secondly, we have determined whether firing rate influences the response of a motoneurone to TMS. We

have re-investigated this question because previous studies by Brouwer, Ashby & Midroni (1989) and Bawa & Lemon (1993) found that, in random mode, response probability was higher at slower firing rates, a finding difficult to explain on the basis of experimental studies of mammalian motoneurons (Schwindt & Calvin, 1972; Baldissera & Gustafsson, 1974).

Finally, we have examined the form of the short-latency response to TMS. This response is made up of a number of subpeaks, which probably reflect the repetitive activation of the corticospinal system by TMS (Day *et al.* 1989; Edgley, Eyre, Lemon & Miller, 1990). The distribution of responses within these different subpeaks should explain how the complex EPSP produced by the descending corticospinal volleys interacts with the changing

†† To whom correspondence should be addressed.

excitability of the motoneurone during the course of the interspike interval.

Part of this work has been the subject of a preliminary publication (Olivier, Bawa & Lemon, 1993).

METHODS

Experiments were carried out on the three authors (32–49 years). Experiments were approved by the local ethical committee. Full details of experimental arrangements are given in Bawa & Lemon (1993). Briefly, surface EMG and single motor unit (SMU) activity were recorded from three muscles: flexor carpi radialis (FCR), flexor digitorum profundus (FDP) and first dorsal interosseous (1DI). Most recordings were from FCR. Surface EMG was recorded with Ag–AgCl electrodes (Arbo), and SMU activity with an intramuscular needle carrying fine (50 μm diameter) stainless-steel recording electrodes.

Procedure

After insertion of the intramuscular electrode, the subject was asked to contract the muscle gently to recruit a clearly identifiable SMU. The subject was given both audio and visual feedback to control the mean interspike interval (ISI). The hand area of the contralateral motor cortex was stimulated with the double-cone coil (diameter 90 mm) of a magnetic stimulator (Magstim 200, 1.5 T). The coil was positioned at the point for which short-latency activation of the gently contracted muscle had the lowest threshold, and the coil was carefully stabilized in this position throughout the experiment (see Bawa & Lemon, 1993).

Two modes of stimulation were used. In one (the random mode), TMS was delivered at random with respect to the discharges of the active SMU. In the other (the triggered mode), the stimulator was triggered by pulses discriminated from the tonically active SMU (see Ashby & Zilm, 1982; Katz, Meunier & Pierrot-

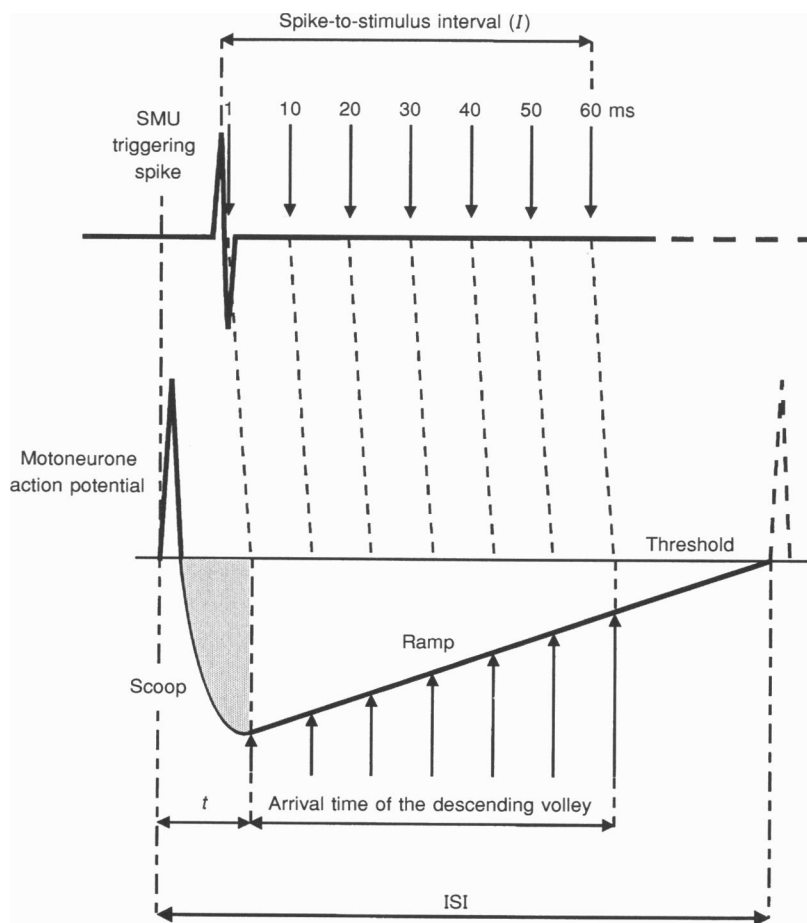


Figure 1. Schematic representation of the trajectory of a motoneurone membrane potential during the interspike interval (ISI)

The time of arrival at the motoneurone of descending volleys (vertical arrows) evoked by TMS when triggered from the spike (upper trace) of a tonically active motor unit. The membrane potential is represented by a 'Scoop' followed by a 'Ramp' according to the data of Schwindt & Calvin (1972). The spike-to-stimulus interval (I) was set at a value between 1 and 60 ms after the motor unit discharge, in 10 ms steps. Because of peripheral and central delays, the arrival of the volley at the level of the motoneurone was $t + I$. For FCR motor units, t was on average 16 ms, which constrained the shortest interval that could be investigated with this method. The shaded area shows the period of time when excitability of the motoneurone could not be tested.

Deseilligny, 1988; Boniface, Mills & Schubert, 1991); the pulses were derived from a double time-amplitude window discriminator, the output of which was monitored throughout the experiment to confirm reliable triggering from the SMU. As shown in Fig. 1, TMS pulses were delivered after a fixed spike-to-stimulus interval, I (1, 10, 20, 30, 40, 50 or 60 ms). After each TMS pulse had been triggered, the stimulator was inactivated for a semirandom period of 3.5–6.0 s before another SMU spike could re-trigger it. With the subject firing the motor unit at a steady and constant rate, TMS was delivered at an interval of 60 ms after the SMU spike. The intensity of the stimulus was adjusted in order to produce a short-latency response from the unit with a response probability typically around 0.6–1.0 at the fast rate. Fifty to one hundred stimuli were delivered for each experimental condition, involving variation of either the SMU firing rate or the spike-to-stimulus interval (I).

The peripheral and central conduction times constrained the minimum delay (t) at which the motoneurone excitability could be tested; the arrival time of the descending volley at the motoneurone was thus $t + I$ ms. For FCR motoneurons, t ranged from 12.9 to 21.6 ms, and was typically around 16 ms. Thus, the excitability of the motoneurone immediately after discharge (the shaded portion in Fig. 1) could not be studied using this method.

Since many of the experiments required recording of the same SMU for long periods, the identity of the unit was repeatedly confirmed during the experiment by inspection of the motor unit triggered average of surface EMG (MU-TA; see Lemon, Mantel & Rea, 1990).

Analysis

Using a 1401 interface and Spike2 software (Cambridge Electronic Design, UK) and a 486 PC, we acquired transistor-transistor logic (TTL) pulses indicating the timing of TMS and SMU discharges, as well as the SMU activity digitized at 10 kHz. TTL pulses associated with SMU spikes were checked for accurate discrimination for the period 200 ms before and after TMS. Any SMU spikes that were not detected by the discriminator (usually because they were superimposed upon spikes of other responding units) were added to the TTL train, while those incorrectly discriminated from other SMUs were excluded. We also confirmed that the SMU interspike interval preceding the delivery of TMS fell within the required fast or slow range. Values are given as means \pm s.d. unless otherwise stated.

RESULTS

Responses of SMUs to TMS

Our analysis is based on recordings from twenty-eight SMUs from the FCR muscle of three subjects; additional observations on one unit in FDP and two in 1DI yielded results which were essentially similar to those from FCR units. The onset latencies of FCR SMU responses to TMS ranged from 12.9 to 21.6 ms. Mean values in the three subjects were 15.7 ± 2.2 , 15.5 ± 0.65 and 19.0 ± 2.1 ms. These latencies, which are similar to those reported previously (Bawa & Lemon, 1993) have not been corrected for the small delays (typically 0.3–1.0 ms) between the onset of motor unit action potential and discriminated TTL pulse. The total duration of short-latency responses

observed in FCR was 4.1 ± 1.9 ms. This value is similar to that reported by Boniface *et al.* (1991) for short-latency responses in 1DI (4.6 ± 1.7 ms).

It is known that the short-latency response of upper limb motoneurons to TMS consists of a number of subpeaks (see Introduction). An example of a motor unit responding with two clear subpeaks to TMS delivered at different delays is shown in Fig. 2A. Two subpeaks were present in the responses of seventeen motor units and six units showed three subpeaks, although their total response duration was not significantly different. In the remaining five motor units, it was impossible to discern subpeaks with any certainty, but again the total response duration was not different from that of the other units with clear subpeaks.

Since the first subpeak is probably elicited by the first of the corticospinal volleys produced by TMS (Day *et al.* 1989; Edgley *et al.* 1990; Burke, Hicks, Gandevia, Stephen, Woodforth & Crawford, 1993), the response probability in this subpeak should provide the most accurate measurement of the excitability of a motoneurone during the interspike interval. However, we were unable to use the response probability of this subpeak for a number of reasons. Firstly, as already mentioned, clear subpeaks were not observed for all motor units. Even for the twenty-three units which did exhibit clear subpeaks, there was a complex relationship between the response probability of the first subpeak and that of the total response. This is illustrated in Fig. 2B and C which compares, for different spike-to-stimulus intervals, the response probability of the total response (filled circles) and that of the different subpeaks for two- and three-subpeak motor units, respectively. A further factor limiting the use of the first subpeak response probability was that, for most SMUs, this subpeak was not present at the shorter intervals (for example see Fig. 2C).

For all of these reasons we decided to use the total response probability as the best practicable measure of motoneuronal excitability during the ISI. In addition, because the distribution of responses within the different subpeaks was strongly influenced by the experimental conditions (see below), this distribution was used, where possible, to give further insights into changes in motoneurone excitability.

Influence of spike-to-stimulus interval on response probability of motor units

As already illustrated in Fig. 2, as the spike-to-stimulus interval was shortened, there was a dramatic fall in the response probability of the total peak. The total peak probability decreased in a monotonic fashion as the interval was shortened, despite the rather complex distribution of response probabilities within the different subpeaks (Fig. 2B and C). Six of the eight SMUs tested at

a spike-to-stimulus interval of 1 ms still responded to TMS at this very short interval.

The progressive decline in the response probability of the total peak as the spike-to-stimulus interval was shortened is further illustrated in Fig. 3 for three different SMUs. The firing rate of each unit and TMS intensity were kept constant during the whole experiment. This monotonic relationship between the spike-to-stimulus interval and the response probability of the total peak over the range of intervals investigated (1–60 ms) was further quantified using regression line analysis. A total of twenty-two series of data were available from thirteen SMUs for which at least four different intervals were tested at constant firing rate and stimulus intensity. For eighteen of twenty-two of these observations (82%) the linear regression between the size of the total peak and the spike-to-stimulus interval was significant ($P < 0.05$). These regression lines have been plotted for the units shown in Fig. 3. The slope of the regression lines for the eighteen significant sets of data had a mean value of $0.012 \pm 0.004 \text{ ms}^{-1}$ (range, $0.005\text{--}0.018 \text{ ms}^{-1}$).

The relative response probability of subpeaks at different spike-to-stimulus intervals

As already shown in Fig. 2, the distribution of SMU responses within the different subpeaks was profoundly influenced by the spike-to-stimulus interval. For the shorter intervals ($<30 \text{ ms}$), the responses falling within the second subpeak (filled squares) represented almost the entire response of the unit to TMS, whereas for the longest interval (60 ms) the response probability in the first subpeak (open squares) accounted for most of the total response. The response probability in the third subpeak (Fig. 2C, open circles) was very small and more or less constant over the range of spike-to-stimulus intervals investigated.

Figure 4A–C shows PSTHs from one unit. At a spike-to-stimulus interval of 60 ms, the response of the SMU was segmented into two subpeaks, with onset latencies of 15.4 and 19.8 ms. At this interval, the relative response probability of the first subpeak (that is the proportion of all responses that fell within this subpeak) was 0.88, while at 30 ms spikes were evenly distributed between the first

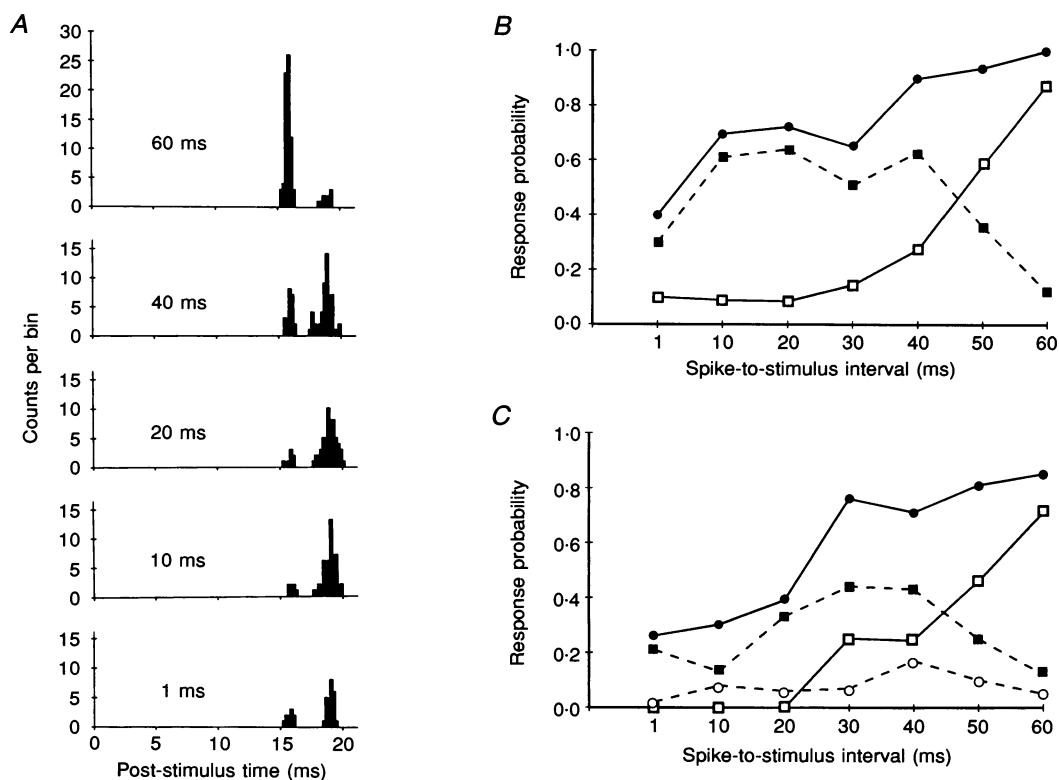
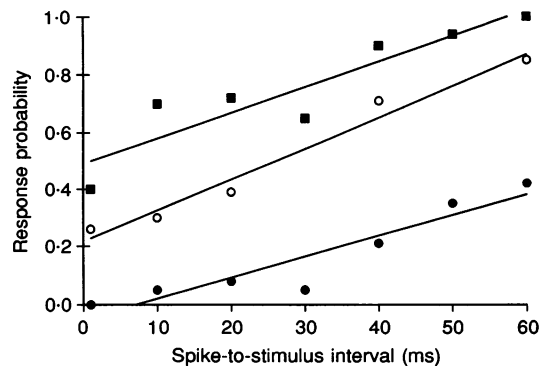


Figure 2. Response probabilities within subpeaks

A, subpeaks in post-stimulus time histograms (PSTHs) of an FCR motor unit. The SMU was tonically firing at about $8 \text{ impulses s}^{-1}$ (slow rate) and TMS was delivered at the different spike-to-stimulus intervals indicated. The latency of the first subpeak was around 15.2 ms. Each PSTH was compiled from about 120 TMS pulses. B and C, relationship between the probability of discharges falling within the total response peak (●) and the probability of those falling within the different subpeaks (□, 1; ■, 2; ○, 3) for a two- (B) and a three-subpeak (C) SMU. Probability is plotted against spike-to-stimulus interval (same SMU as shown in A).

Figure 3. Relationship between the probability of the total peak response and spike-to-stimulus intervals for three different FCR motor units

Lines represent regression lines computed for the following three sets of data. ■: SMU tested at slow rate, $r = 0.93$, $P = 0.001$; ○: SMU tested at fast rate, $r = 0.91$, $P = 0.003$; ●: SMU tested at slow rate, $r = 0.96$, $P = 0.001$.



and second subpeaks with relative response probabilities of 0.53 and 0.47, respectively (Fig. 4B). At the shortest interval (1 ms), no first subpeak was present and the few responses obtained were all within the second subpeak (Fig. 4C).

The relative response probabilities of the different subpeaks as the interval was shortened have been plotted in Fig. 4D and E for a two- and a three-subpeak unit, respectively. The data illustrated in Fig. 4A–D are from the same unit. The relative response probability of the

first subpeak decreased progressively as the spike-to-stimulus interval shortened, and the proportion of responses found within the second subpeak increased. For the three-subpeak SMU (Fig. 4E), the progressive decrease in the first subpeak was accompanied by a monotonic increase in the third, while the second subpeak first increased and then decreased as the spike-to-stimulus interval was shortened.

As the probability of the first subpeak fell with the shorter spike-to-stimulus interval, the latency of the

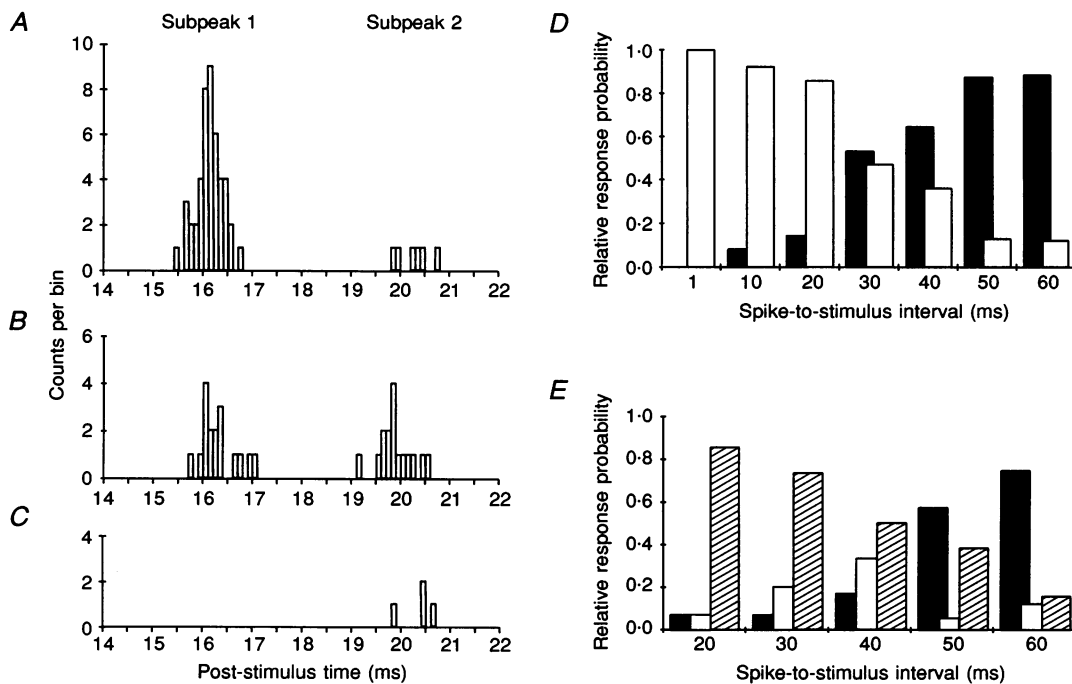


Figure 4. Influence of spike-to-stimulus interval on the distribution of responses within different subpeaks

Distribution of subpeaks at 60 (A; response probability for total peak, $P = 0.85$), 30 (B; $P = 0.44$) and 1 ms (C; $P = 0.06$) spike-to-stimulus intervals for a two-subpeak FCR motor unit firing at fast rate (about 12 impulses s^{-1}). Each PSTH was compiled from about 85 TMS stimuli. D, histograms indicating change of relative response probability of response in different subpeaks (■, 1; □, 2) with respect to the spike-to-stimulus interval (same SMU as in A–C). E, relative response probabilities for a three-subpeak (■, 1; □, 2; ▨, 3) FCR motor unit tested as slow rate (around 8.7 impulses s^{-1}).

earliest response within this subpeak became slightly longer. The difference in the latency of the first subpeak obtained for a given SMU at the shortest and the longest spike-to-stimulus intervals investigated (0.65 ± 0.73 ms, 20 SMUs, 37 pairs of observations) was statistically significant (Student's paired t test, $t = 5.35$, $P < 0.001$). This increase in latency was associated with a significant decrease in the duration of the first subpeak (0.62 ± 0.90 ms; paired t test, $t = 4.16$, $P < 0.001$). Neither the latency nor the duration of the second subpeak showed any significant change as the spike-to-stimulus interval was shortened.

The effect of firing rate on responses to TMS in the triggered mode

When TMS was delivered in the triggered mode, at a given interval, every unit studied had a greater response probability at the faster firing rate. This finding is illustrated by the PSTHs from one unit in Fig. 5. The distribution of interspike intervals (ISI) at the fast and slow firing rates is shown by the interspike interval

histograms (Fig. 5, insets). For a spike-to-stimulus interval of either 40 or 10 ms, the total peak response probability was higher at the faster firing rate (ISI mode around 75 ms; Fig. 5A and B) than at the slower rate (ISI mode around 110 ms; Fig. 5C and D). Of a total of sixteen SMUs tested at two different firing rates for the same spike-to-stimulus interval (52 paired comparisons), the response probability at the faster rate (mean modal ISI: 83 ± 9.6 ms) was systematically higher than at the slower rate (mean modal ISI: 116 ± 15 ms). This difference was highly significant (paired t test, $t = 10.4$, $P < 0.001$).

In order to compare the time course of the change in response probability at fast and slow rates, the response probabilities of seven SMUs were calculated for multiple spike-to-stimulus intervals (between four and seven) at both rates. The effects of firing rate were consistent for all SMUs; the data from three of them are illustrated in Fig. 6. Whereas at fast rate SMUs still responded at intervals as short as 1 ms (albeit with low response probabilities), at the slower rate response probability fell to zero at longer intervals.

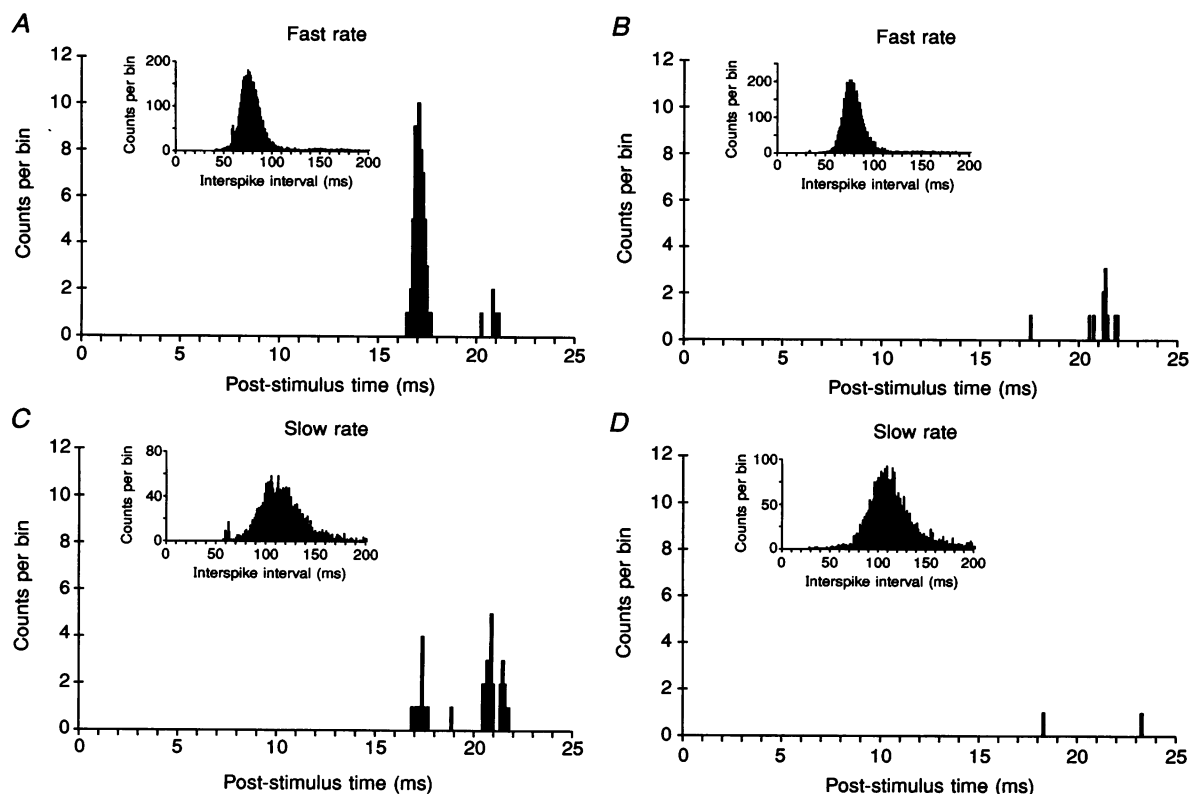
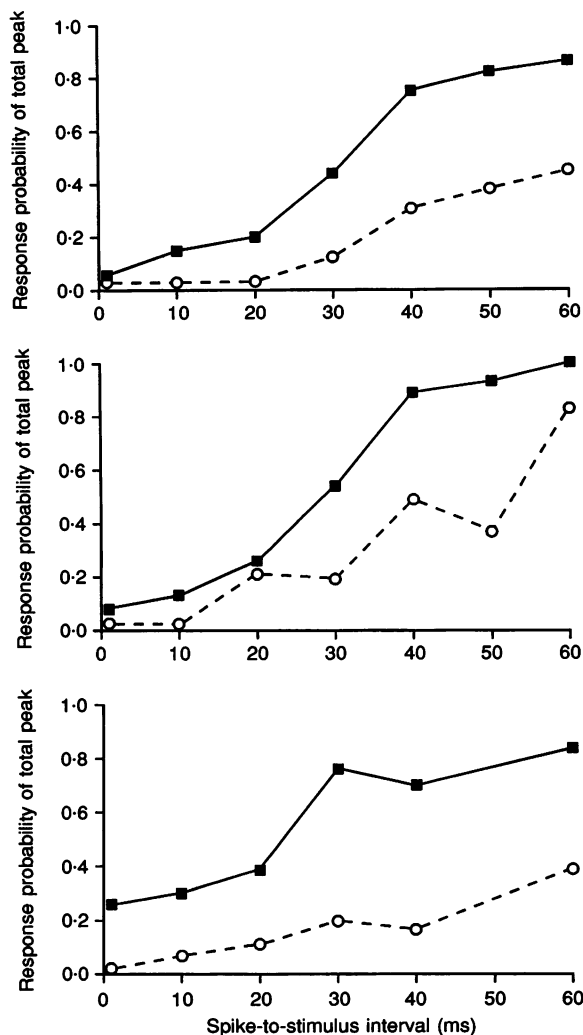


Figure 5. Effect of firing rate on the response probability of an FCR motor unit when TMS was delivered in the triggered mode

PSTHs computed for the same SMU tested at two different spike-to-stimulus intervals (40 (A and C) and 10 ms (B and D)) at fast (A and B; around $13 \text{ impulses s}^{-1}$) and slow rates (C and D; around $9 \text{ impulses s}^{-1}$). For A, the number of TMS stimuli used to compile the PSTHs ($n = 74$, response probability of the total peak ($P = 0.89$); for B, $n = 87$, $P = 0.13$; for C, $n = 72$, $P = 0.50$; and for D, $n = 118$, $P = 0.02$). Insets show interspike interval histograms computed for each experimental condition. Note the different ordinate scales. On the interspike interval histograms shown in A and C, the peak response to TMS is visible to the left of the main distribution.

Figure 6. Effects of firing rate on response probabilities of three SMUs at multiple phases of ISIs

Relationship between total peak probability and spike-to-stimulus interval at the fast (> 12 impulses s^{-1} , ■) and slow rates (< 10 impulses s^{-1} , ○) for three different FCR motor units.



The firing rate also influenced the distribution of the responses within the different subpeaks as shown in Fig. 7. The relative probability of responses within the first subpeak was higher at the fast rate than at the slow rate (paired *t* test, $n = 46$, $t = 5.6$, $P < 0.001$). The converse was seen for the second and third subpeaks i.e. the relative probability of the spikes falling within these subpeaks was higher at the slow rate than at the fast rate (paired *t* test, second subpeak: $n = 46$, $t = 4.02$, $P < 0.001$; third subpeak: $n = 20$, $t = 1.83$, $P = 0.04$).

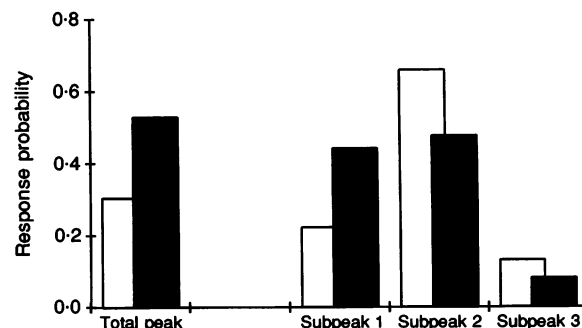
Figure 7. Summary of the effects of firing rate on response probabilities in the triggered mode

The absolute response probability of the total peak (left) and the relative response probability of the different subpeaks (right) at slow (□) and fast (■) firing rates. This was computed for all values of spike-to-stimulus interval ($n = 52$) from sixteen FCR motor units.

This difference was observed irrespective of the spike-to-stimulus interval.

Comparison of responses to triggered and random modes of stimulation

Six SMUs were tested in both the triggered mode, as described above, and with TMS stimuli delivered at random with respect to the SMU discharges. In the random mode, five of six SMUs had a larger response probability at the slower firing rate; this difference was statistically significant (paired *t* test, $t = 2.4$, $P = 0.03$).



This finding is illustrated by the PSTHs from one unit tested at two different firing rates under random conditions (Fig. 8*A* and *B*).

Only three units which were tested with the full range of spike-to-stimulus intervals, and which therefore bore the closest resemblance to the random mode of stimulation, could be used to compare response probability in both the random and the triggered mode. The mean results for the three units are plotted in Fig. 8*C*; the responses have been normalized to the response probability found at the slow rate in the random mode (100%). In this mode, responses at the fast rate were marginally smaller (92%). In the triggered mode, the mean response probability at the slow rate, pooled for all intervals tested, was less than half that at the slow rate in the random condition (48%). The difference between response probabilities at slow and fast rate was much larger than in the random mode.

Figure 9 shows the relationship between response probability and spike-to-stimulus interval when data

obtained in the random mode were collected into 10 ms bins to mimic the triggered mode. The advantage of this approach is that it allows estimation of the response probability of the motoneurone for the entire duration of the ISI. Figure 9 clearly shows the sigmoidal shape of the relationship between response probability and spike-to-stimulus interval. At the faster rate, the sigmoidal curve relating response probability to spike-to-stimulus interval was systematically shifted to the left. In addition, this confirms that the response probability over the 1–60 ms range investigated in the triggered mode was always higher at the fast rather than at the slow rate. The change in response probability over this range can be approximated by a linear function (dotted lines) which supports the use of linear regression analysis of data obtained in the triggered mode.

The distribution of the responses within the different subpeaks was also influenced by the mode of TMS delivery. For the three units, the relative probability of responses within the first subpeak was lower in the

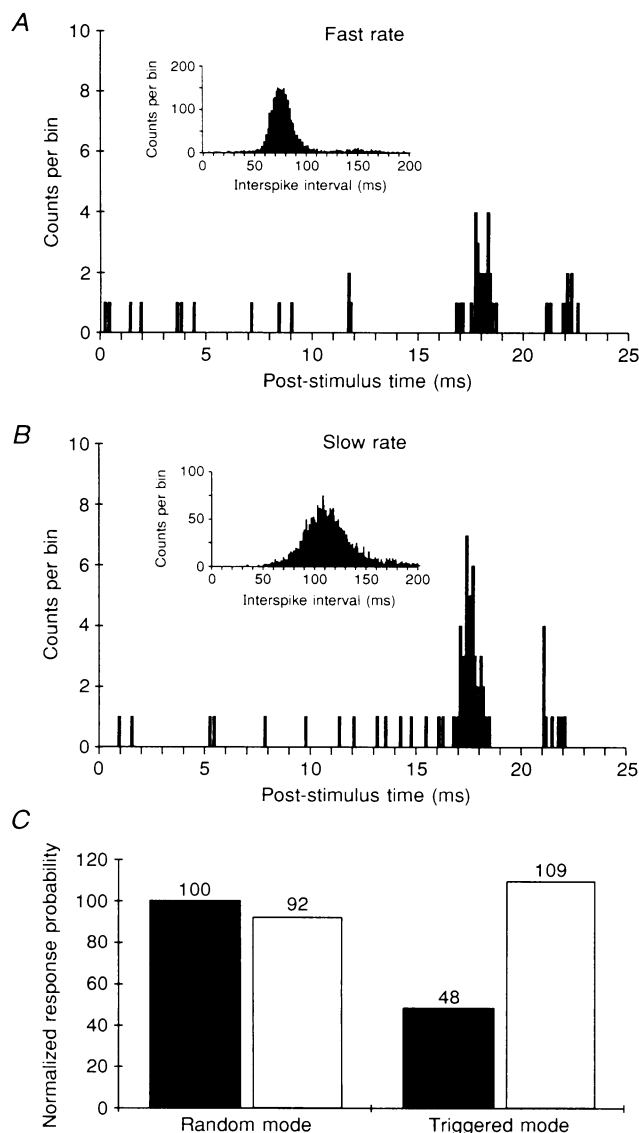


Figure 8. Effect of firing rate on response probability when TMS was delivered randomly with respect to voluntary discharge

PSTHs computed for the same FCR motor unit tested at fast (*A*; $n = 81$, $P = 0.37$) and slow (*B*; $n = 108$, $P = 0.45$) firing rate. Insets show interspike interval histograms computed for each experimental condition. Note the different scales on the ordinates. Data are from the same unit as shown in Fig. 5. *C*, comparison of rate effect (□, fast; ■, slow) on response probability of the total peak in the random and in the triggered mode for three different FCR motor units. Response probabilities have been normalized with respect to that obtained at slow rate in the random mode of stimulation (100%; column on extreme left).

triggered mode (0.37) than in the random mode (0.74), irrespective of the firing rate (paired *t* test, $n = 6$, $t = 3.9$, $P < 0.01$).

DISCUSSION

This study shows that the excitability of a rhythmically firing motoneurone, as revealed by its responses to TMS, undergoes substantial changes during the interspike interval.

Use of transcranial magnetic stimulation to assess motoneuronal excitability

It is known that TMS of the motor cortex generates several successive volleys in the corticospinal tract (Day *et al.* 1989; Edgley *et al.* 1990; Mills, 1991; Edgley, Eyre, Lemon & Miller, 1992; Burke *et al.* 1993). As a consequence, EPSPs underlying motoneurone responses to TMS are complex (S. A. Edgley, J. A. Eyre, S. Miller & R. N. Lemon, unpublished observations). This is confirmed by the presence of subpeaks in the short-latency responses of upper limb motor units (Day *et al.* 1989; Boniface *et al.* 1991; Bawa & Lemon, 1993).

In this study, since motoneuronal excitability was estimated by using the response probability of the total peak, the relationship between response probability and spike-to-stimulus interval results from the action of a complex EPSP. Indeed, for the longer spike-to-stimulus intervals, the earliest component of the EPSP generated by the first descending volley was generally sufficient to discharge the motoneurone, as revealed by the high

probability of discharge in the first subpeak. For shorter spike-to-stimulus intervals, when the motoneurone is less excitable, temporal summation and facilitation by the successive corticospinal volleys would be necessary to produce an EPSP large enough to bring the motoneurone to threshold. This mechanism would explain why it was possible to excite motoneurones at spike-to-stimulus intervals as short as 1 ms. At these short intervals, the time course of this complex EPSP would also result in most of the motoneurone discharge falling in the second or third subpeak, and this is what we observed.

Theoretically, the relationship between the total peak probability and spike-to-stimulus interval is the same as would be obtained in response to the larger and later component of the EPSP responsible for the SMU discharges falling within the late subpeaks. This explains why such a complex distribution of SMU responses (see Fig. 2) in different subpeaks nevertheless gave rise to a linear relationship between total peak probability and spike-to-stimulus interval. A similar analysis based on the response probability of the first subpeak (SP1) would allow investigation of the response of motoneurones to the first EPSP. This should lead to a shift to the right of the sigmoidal curve relating response probability to interval.

The use of the total response probability as an index of the excitability of the motoneurone, however, introduced a small error in the estimate of the shortest interval at which the descending volley was able to bring a motoneurone to threshold. This error is due to the fact that the latency of SMU responses is not constant but

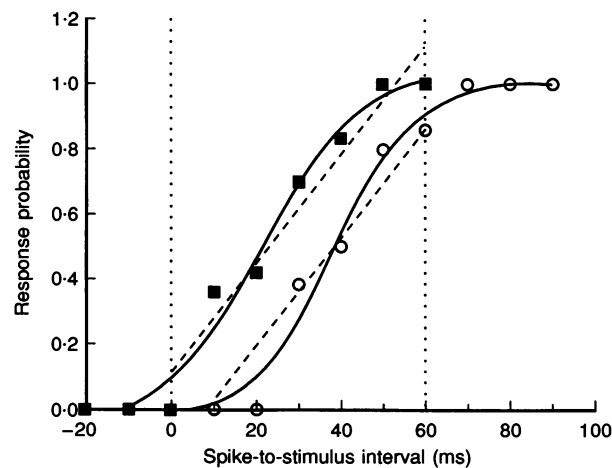


Figure 9. Relationship between the total response probability and spike-to-stimulus interval at the fast (> 12 impulses s^{-1} , ■) and slow rates (< 10 impulses s^{-1} , ○) in the random mode

The spike-to-stimulus intervals have been collected into 10 ms bins over the whole interspike interval. Spike-to-stimulus intervals are expressed with respect to the motor unit discharge, therefore negative values represent cases in which magnetic stimulation fell before the motor unit fired spontaneously, within an interval smaller than the latency of the motor unit to TMS. Continuous lines represent the sigmoid function fitted over the whole range of intervals. Dashed lines are regression lines computed over the same range of intervals as that investigated in the triggered mode and delimited by vertical dotted lines (1–60 ms). Both regression lines are significant ($P < 0.05$); coefficients of correlation are 0.97 and 0.95 at the fast and the slow rate, respectively.

depends on the spike-to-stimulus interval. As we have already emphasised above, for shorter intervals and particularly at the slow rate, SMU responses fell mainly within the second or third subpeak, adding an extra central delay of a few milliseconds to the SMU response latency. This led to an underestimate of the minimal time at which the descending volley was able to bring motoneurons to threshold and, therefore, to an underestimate of the slope of regression lines relating response probability and interval. However, this error cannot be greater than the duration of the total peak (4.1 ± 1.9 ms) and its influence on our results is thought to be negligible.

Relationship between response probability of rhythmically firing motoneurons and spike-to-stimulus interval

In a noise-free system, the relationship between the response probability and spike-to-stimulus intervals would be a step function with probability varying from 0 to 1

instantaneously, when the EPSP amplitude becomes larger than the difference between spike threshold and membrane potential. However, taking into account the synaptic noise and the variability of the descending excitatory inputs, this relationship is better approximated by a sigmoidal function. This assumption is supported by experiments using Ia inputs (Piotrkiewicz, Churikova & Person, 1992, Fig. 3), as well as by our data obtained with TMS delivered at random with respect to the previous spike (Fig. 9). Besides the 'noise' of the system, the slope of the rising phase of the sigmoidal curve will also depend upon the amplitude and shape of the AHP, as well as changes in membrane conductance and spike threshold during the interspike interval.

In this study our approach has been to adjust carefully the intensity of TMS such that the probability of the responding unit at the fast rate was typically between 0.6 and 1.0 at a spike-to-stimulus interval of 60 ms. Because

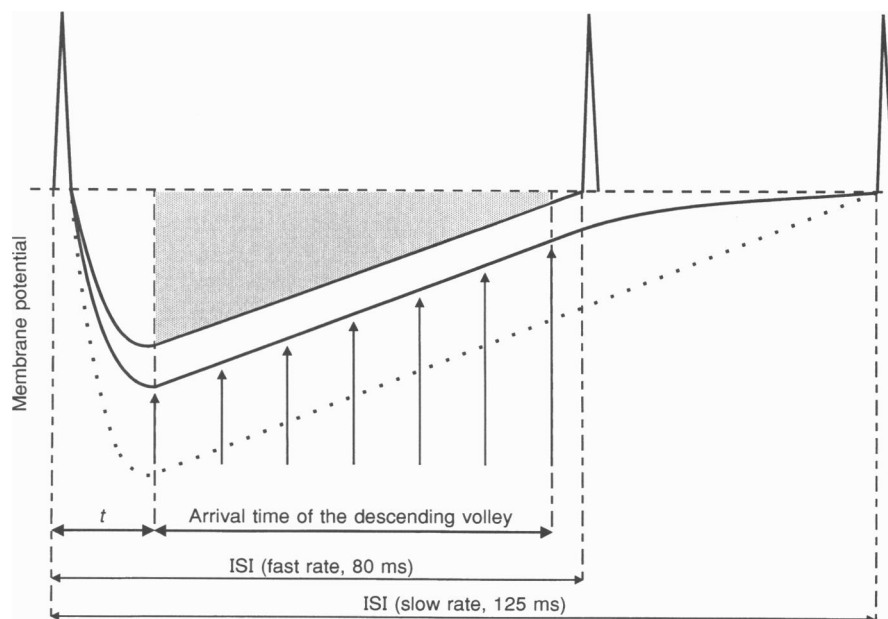


Figure 10. Schematic representation of the trajectory of a motoneurone membrane potential during the interspike interval (ISI) at slow and at fast rate which might explain the reversal of the rate effect depending on the mode of TMS delivery

Dotted membrane potential trajectory at the slow rate represents the trajectory predicted by the data of Schwindt & Calvin (1972) on cat motoneurons. The fast rate was arbitrarily set at $12.5 \text{ impulses s}^{-1}$ (ISI, 80 ms) and the slow rate at $8 \text{ impulses s}^{-1}$ (ISI, 125 ms). In the triggered mode the period of ISI investigated was between $t + 1$ and $t + 60$ ms (shaded portion). At the slow rate this period amounted to 48% of the ISI, whereas at the fast rate it corresponded to about 75% of the ISI. Assuming a constant descending volley, the higher response probability obtained at the fast rather than at the slower rate can therefore be explained by a shallower AHP at the fast rate. In the random mode of stimulation, the period of the ISI investigated corresponds to the whole duration of the ISI and the Schwindt & Calvin data (1972) (dotted line) predicts that a higher response probability should still be obtained at the fast rate. To explain the higher response probability at the slow rate in the random condition, the initial part of the ISI during which the motoneurone excitability is lower must be compensated for by a period of higher excitability in the later part of the ISI. The diagram suggests that this results from the trajectory of the membrane potential at the slow rate, in which it approaches the threshold voltage in an asymptotic manner, leading to a prolonged increase in the motoneuronal excitability towards the end of the ISI.

we were successfully able to apply linear regression analysis to data obtained for the 1–60 ms intervals investigated in the triggered mode, this period probably corresponds to the rising phase of the sigmoidal curve. Any change in TMS intensity or firing rate should, however, lead to a shift of the sigmoidal curve and therefore modify the interval over which the relationship between response probability and intervals can be approximated by a linear function. For example, a reduction in the voluntary SMU firing rate led to a shift of the sigmoidal curve to the right (Fig. 9).

Effect of the firing rate on the response probability in the triggered mode

In the triggered mode, the probability for a given SMU of responding to TMS was systematically higher at the faster firing rates than at slower rates. This result can be predicted from data available on the membrane potential trajectories of cat lumbar motoneurones firing at different rates. In motoneurones made to discharge rhythmically by step current injection, the trajectory of the interspike membrane potential has been described by a 'scoop' followed by a 'ramp' (Schwindt & Calvin, 1972; Baldissera & Gustafsson, 1974; Calvin, 1975). Within the primary range (< 35 impulses s^{-1}), Schwindt & Calvin (1972) found that both the amplitude and the duration of the 'scoop' are linear functions of the interspike interval and that the 'ramp' can be approximated by a straight line with a constant slope, irrespective of the firing rate. As a consequence, the after-hyperpolarization (AHP) is always shallower at the faster rate. This is shown diagrammatically in Fig. 10. Therefore, assuming that the shape of the membrane potential trajectory is a major determinant of the response probability of a rhythmically firing motoneurone, these data predict that the excitability of the motoneurone should always be higher at faster than at slower rates.

However, this conclusion is based on a questionable assumption that the other factors determining the excitability of the motoneurone (synaptic noise, membrane conductance and firing threshold) are not affected by changes in firing rate. It must further be stated that, while the described changes in response probability during the ISI are generally consistent with the AHP in cat motoneurones recorded by Schwindt & Calvin (1972), it would be difficult to explain if the AHP amplitude in an active motoneurone was very small, as suggested by Brownstone, Jordan, Kriellaars, Noga & Schefchyk (1992).

A further assumption is, of course, that there are no significant changes in the amplitude of EPSPs evoked by the descending corticospinal volley at the different firing rates. A change in amplitude of cortico-motoneuronal EPSPs evoked by TMS may occur since many studies show an increased level of corticospinal activity at higher force levels (Cheney & Fetz, 1980; Evarts, Fromm,

Kröller & Jennings, 1983; Maier, Bennett, Hepp-Reymond & Lemon, 1993), and it has been argued that TMS produces a descending volley that, in part, reflects the level of cortical activity (Day, Riescher, Struppler, Rothwell & Marsden, 1991; Flament, Goldsmith, Buckley & Lemon, 1992). However, at present, there is no direct evidence that variation in cortical activity, related to gradations in force, results in a larger corticospinal response to TMS. Nor is there any evidence concerning the extent to which this factor might contribute to the rate-related changes we have observed. In particular, it is noteworthy that similar rate effects have been recently documented by Piotrkiewicz *et al.* (1992) and Jones & Bawa (1993) using Ia inputs to the FCR motoneurone pool. It would thus appear reasonable to assume that the changes we have observed with TMS reflect changes in motoneuronal excitability rather than changes in EPSP amplitude evoked by corticospinal descending volleys.

Effect of the firing rate on the response probability in the random mode

A striking finding of the present study is that, whereas a markedly higher response probability was found at faster rate in the triggered mode of stimulation, this contrasted with the slightly higher probability at the slow rate in the random mode (see also Brouwer *et al.* 1989; Bawa & Lemon, 1993).

This result strongly suggests that, for the early part of the ISI, which we investigated in the triggered mode, the fraction of the ISI during which motoneurones are excitable is larger at the faster rate, and this is supported by the cat motoneurone data (Schwindt & Calvin, 1972; Baldissera & Gustafsson, 1974). Therefore, the only explanation for the reversal of the 'rate effect' in the random mode is that, at the slow rate, the initial period of low response probability is followed by a period of high motoneuronal excitability. Of course this late period, beginning around 60 ms after SMU discharge, was not investigated in the triggered mode but in the random mode, it would compensate for the reduced level of motoneuronal excitability observed during the early part of the ISI at slower firing rates. This late period of high excitability could be explained by the membrane potential remaining very close to the firing threshold throughout the late part of the ISI (see Fig. 10). Examples of the membrane potential following such an asymptotic trajectory can be seen in recordings from cat motoneurones firing rhythmically at very slow rates (Schwindt & Calvin, 1972; Baldissera & Gustafsson, 1974; Calvin, 1974, 1975). A similar feature has been suggested by Ellaway & Murthy (1985) to explain the discharge patterns of cat γ -motoneurones (see also Tuck, 1977).

Thus, the lower response probability seen at the slow rate in the triggered mode could be explained by the fact that motoneurone excitability was only tested over the initial part of the ISI when the difference between the spike

threshold and membrane potential is larger at the slow rather than at the fast rate (see Fig. 10). This hypothesis was confirmed when SMU responses to TMS delivered at random were collected into 10 ms bins and estimated over the same period of time as in the triggered mode (see Fig. 9). At the slow rate, this initial period of lower response probability was followed by a relatively long period during which the response probability was close to 1. We suggest that it is this feature which explains the present results, and those reported previously by Brouwer *et al.* (1989) and Bawa & Lemon (1993).

The corollary of this hypothesis is that, since the amount of time over which an EPSP can bring the motoneurone to threshold is larger at slow firing rate, the regularity of motoneurone discharge, as estimated by the coefficient of variation of ISI, should increase as the firing rate decreases (Ellaway, 1972; Ellaway & Murthy, 1985). This is supported by our data showing that interspike interval histograms were more dispersed for motor units firing at slow rates (see Figs 5 and 8). Similar results can be extrapolated from data published by Kranz & Baumgartner (1974, see their Fig. 4).

In conclusion, our results with TMS demonstrate that during the interspike interval of rhythmically firing human motoneurons there is an initial increase in response probability that is linearly related to the spike-to-stimulus interval, followed by a later phase in which response probability asymptotes. There are significant differences in response probability at slow and at fast discharge rates.

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