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Age and sex differences in human motor cortex input–output characteristics

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Stimulus–response curves for motor evoked potentials (MEPs) induced in a hand muscle by transcranial magnetic stimulation (TMS) were constructed for 42 subjects with the aim of identifying differences related to age and sex. There was no effect of age on the resting threshold to TMS, the maximal amplitude of the MEP that could be evoked (MEP_{max}) or the maximal slope of the stimulus–response curve. However, higher stimulus intensities were required to achieve both MEP_{max} and the maximal slope in the older subjects. The trial-to-trial variability of MEPs was greater in the older subjects, particularly at intensities near threshold. There was a significant interaction between age, threshold and trial-to-trial variability of MEP amplitude. Overall, MEP variability fell markedly as stimulus intensity increased above threshold but less rapidly in older than in younger subjects. Females tended to have larger MEP variability than males, but age and threshold were much stronger modulators than sex. These differences in input–output characteristics are likely to be due either to a decreased number of spinal motoneurones being activated synchronously in older subjects, or to the activation of the same number of motoneurones in a less synchronous manner, leading to phase cancellation in the surface electromyogram.

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Transcranial magnetic stimulation (TMS) is widely used to study the properties of corticospinal pathways. In recent years, it has also been used to study cortical reorganization in response to interventions such as amputation, afferent stimulation, motor learning, cortical and spinal lesions, ischaemia and limb immobilization (reviewed in Cohen *et al.* 1998). However, we have recently noted that the amount of plastic change in response to both muscle fatigue (Pitcher & Miles, 2002) and peripheral nerve stimulation (J. B. Pitcher, M. C. Ridding & T. S. Miles, unpublished observations) appears to be influenced by the amplitude of the motor potential evoked (MEP) by TMS prior to experimental intervention. This suggests that the ability of the motor cortex to reorganize may depend on its input–output characteristics, the nature of which appears to differ markedly between individuals. In addition, our observations suggested that these differences might be further influenced by subject sex and age. Therefore, the aim of this study was to compare the input–output characteristics of the motor cortex of human male and female subjects, and the effect of age on these characteristics.

METHODS

Twenty male and 22 female subjects (age range 18 to 55 years, mean ± S.D. = 28.7 ± 10.5 years) gave informed written consent to participate in the study. Subjects had no relevant medical history. All investigations were performed in accordance with the Declaration of Helsinki. Ethical approval was obtained from the Human Research Ethics Committee of The University of Adelaide.

Stimulation and EMG recording

Subjects were seated with their right hand and forearm supported. Surface electromyograms (EMG) were recorded from the first dorsal interosseous (FDI) muscle of the right hand with one silver–silver chloride electrode placed over the motor point and the other over the metacarpophalangeal joint. MEPs were recorded only when the EMG indicated that the FDI was inactive. Myoelectric signals were digitized (2.1 kHz) and stored on a 1401 laboratory interface (Cambridge Electronic Design, Cambridge, UK) for off-line analysis.

Single-pulse TMS were delivered by a Magstim 200 magnetic stimulator (Magstim Co., Dyfed, UK) with a figure-of-eight stimulating coil over the area of the left motor cortex that was optimal for producing MEPs in the right FDI. The MEP threshold was defined as the lowest stimulator output at which five MEPs with minimum peak-to-peak amplitude of 50 μV were evoked from the resting FDI in ten consecutive trials.

In 16 of the subjects (8 females), 10 consecutive maximal M-waves were evoked in the FDI at 4 s intervals by single, supra-maximal stimuli through surface electrodes over the ulnar nerve at the wrist. This was performed prior to recording the input–output curve.

Protocol

An input–output curve for the MEP amplitude evoked in the resting FDI by TMS was constructed. Ten TMS were delivered at each intensity, beginning 10–12 % below resting threshold and increasing incrementally in 3 % steps to either 100 % of stimulator output, or to a stimulus intensity where MEP amplitude had

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Table 1. MEP and M-wave characteristics (means ± S.D.) for male and female subjects

Parameter	*n*	Sex	Mean ± S.D.
Age (years)	22	Females	28.3 ± 11.0
	20	Males	29.0 ± 10.3
Resting threshold (% stimulator output)	22	Females	39.6 ± 8.1
	20	Males	41.6 ± 8.2
MEP_{max} (mV)	22	Females	2.1 ± 1.8
	20	Males	1.7 ± 1.2
Slope of input–output curve	22	Females	0.17 ± 0.05
	20	Males	0.11 ± 0.05
Range (mV) (threshold to MEP_{max})	22	Females	2.2 ± 1.5
	20	Males	1.9 ± 1.6
Range (% stimulator output)	22	Females	31.5 ± 7.8
	20	Males	31.4 ± 7.7
M-wave (mV)	8	Females	17.5 ± 4.7*
	8	Males	12.7 ± 3.5
MEP max% ([MEP_{max}/M-wave]%)	8	Females	14.9 ± 12.5
	8	Males	21.2 ± 14.0

* $P = 0.04$ when compared with males.

reached a plateau. The MEPs evoked at each stimulus intensity were averaged on-line. The peak-to-peak amplitude and onset latency of these averages were then determined with custom-written software (LabVIEW, National Instruments, Austin, TX, USA). All MEP and M-wave data are presented as the ensemble averages of 10 trials.

The amplitudes of the MEP averages were plotted against stimulus intensity in each subject, and the Marquardt-Levenberg algorithm for least squares convergence (Sigmaplot for Windows 5.0, 1986–1999; SPSS Inc., IL, USA) was used to calculate the best fit of the cortical stimulus–response curves of each subject. The calculation was based on the highest coefficient of determination, R^2, and the resulting 5-parameter sigmoidal curve is described by the following equation:

$$f = y_0 + a/(1 + \exp(-(x - x_0)/b)) \wedge c, \quad (1)$$

where the five parameters are the difference between the smallest and the largest MEP amplitudes observed, i.e. the range between the two asymptotes (a); the minimum MEP amplitude (y_0); the difference between the stimulus intensities at 75 % and 25 % of the maximum MEP amplitude (i.e. the width of the transition) (b); the stimulus intensity required to obtain an MEP that is 50 % of the maximum MEP amplitude (x_0); and the slope constant (c). The constraints of the relationship are that $c > 0$ and that $y_0 = 0$. The predicted resting threshold (x), i.e. percentage stimulator output, when $y = 50$ μV was derived from eqn (1) as follows:

$$x = x_0 - b \ln[((a/y - y_0) \wedge (1/c)) - 1]. \quad (2)$$

The slope of the stimulus–response curve (y') was determined as:

$$y' = c/b \times (1 - (1/g(x)) \times (y - y_0). \quad (3)$$

Finally, the rate of change of the slope at each stimulus intensity (y'') was computed as:

$$y'' = c/b \times (1 - 1/g) \times (y' - ((y - y_0)/(b \times g)), \quad (4)$$

where $g = 1 + \exp((x - x_0)/b)$

The maximum MEP amplitude (MEP_{max}) was the largest peak-to-peak, ensemble-averaged MEP amplitude recorded. MEP_{max} was also calculated as a percentage of the maximum M-wave recorded in that subject (i.e. MEP_{max}/M_{max}).

The MEP data were analysed with univariate analysis of variance (ANOVA; SPSS for Windows v.10.0.5, SPSS Inc., 1989–1999). Specific comparisons (e.g. on the basis of age, sex, resting threshold, etc.) were made using covariate analyses. Estimates of effect size were computed using the partial eta squared (η_P^2) statistic to determine the influence of age and sex on curve variables. In addition, the coefficients of variation (CV) of MEP amplitude and M-wave amplitude were calculated from the mean amplitude and standard deviation of the ten consecutive individual MEPs or M-waves (i.e. not the ensemble average) recorded at each intensity in each subject. The MEP CV data were analysed using between- and within-factor repeated measures analysis of variance, with specific contrasts (ANOVA; SPSS for Windows v.10.0.5, SPSS Inc., 1989–1999). The within-factor was stimulus intensity and the between-factor was sex (two levels: male and female). *Post hoc* analyses were carried out using Bonferroni's comparison with corrections. Relationships between variables were assessed by computing Pearson's product–moment correlation coefficient. All comparisons and correlations were two-tailed. Statistical significance was assumed at $P \leqslant 0.05$.

RESULTS

Although there was considerable variation between subjects in cortical stimulus–response characteristics, the stimulus–response data were fitted best by the 5-parameter sigmoid in all but 4 of the 42 subjects. For these 38 curves, the coefficients of determination were all greater than 0.90 (i.e. $R^2 \geqslant 0.90$). An example of the averaged MEP responses and stimulus–response curve of one subject is shown in Fig. 1. The remaining four subjects' data were best fitted by a 4-parameter sigmoid; however, when fitted with the 5-parameter sigmoid, the coefficients of determination in these four subjects were still greater than 0.80. Hence, all data are presented as curves based on the 5-parameter analysis.

The measured resting threshold for each subject was highly correlated with the resting threshold predicted by the algorithm (Pearson's $r = 0.90$, $P \leqslant 0.0001$, $n = 42$). Similarly, the measured MEP_{max} was highly correlated with the predicted MEP_{max} (Pearson's $r = 0.86$, $P \leqslant 0.0001$, $n = 42$). Neither the measured nor the predicted resting thresholds changed with subject age. While females tended to have lower resting thresholds than males, this difference was small (2–3 % stimulator output) and not significant. The means and standard deviations of the key variables for all male and female subjects are shown in Table 1.

Effect of age

The peak slope of the fitted stimulus–response curve was not different in older compared with younger subjects, but the curve itself tended to be shifted to the right. That is, higher stimulus intensities were required to evoke equivalent amplitude MEPs in older subjects. The stimulus–response

curves up to MEP_{max} of two representative subjects aged 46 and 29 years and the curve slopes at each stimulus intensity are shown in Fig. 2. These subjects had similar resting thresholds, MEP_{max} amplitudes and maximal slopes (Fig. 1*B*), but in the older subject the rate of slope change was slower (i.e. the rate at which MEP amplitude increased with increasing stimulus intensities was slower) which shifted the stimulus–response curve to the right. Across the population, the maximal slope of the input–output curve did not alter with age (Fig. 3*A*) and was not different when genders were compared. However, as subject age increased, so did the stimulator output required to elicit 50 % MEP_{max} (Age, $P = 0.02$, $n = 42$) and MEP_{max} (Age, $P = 0.05$, $n = 42$).

Both the TMS intensity required to evoke 50 % MEP_{max} and the maximal rate of slope increase were correlated with higher resting thresholds (Threshold, $P = 0.01$, $n = 42$); however, because there was no interaction between age and increasing resting threshold, the changes seen in older subjects were not the result of increased resting thresholds. Figures 2*B* and 3 show that there was a marked increase

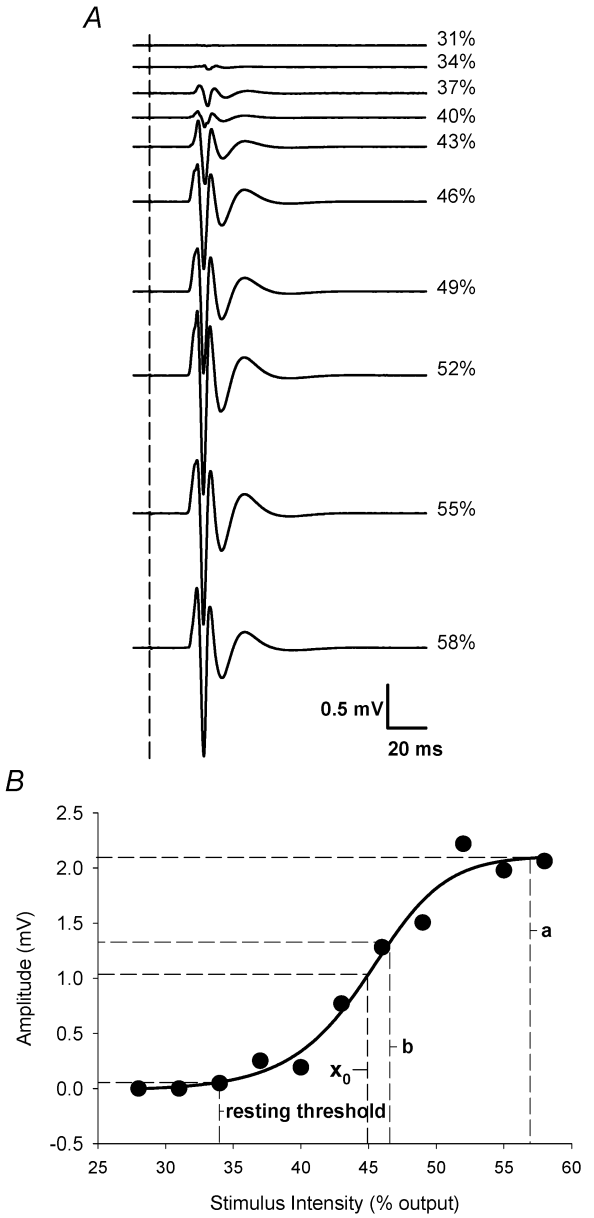

Figure 1. The stimulus–response (TMS–MEP) relationship for resting FDI in one subject

A, averaged MEPs ($n = 10$) recorded across the range of responses evoked at different TMS intensities expressed as percentage of maximal stimulator output. *B*, the stimulus–response curve for this subject, based on peak-to-peak amplitude of averaged MEPs at each TMS intensity. Data are ensemble averaged MEPs at each given stimulus intensity. The points were best fitted by a 5-parameter sigmoid derived using the Marquardt-Levenberg algorithm ($R^2 = 0.98$). Drop lines indicate the stimulator output at resting threshold (34 %), the stimulus intensity required to obtain an MEP that is 50 % of the maximum MEP amplitude (x_0), difference between the stimulus intensities at 75 % and 25 % of maximum MEP (b), and the MEP_{max} predicted by the algorithm (a).

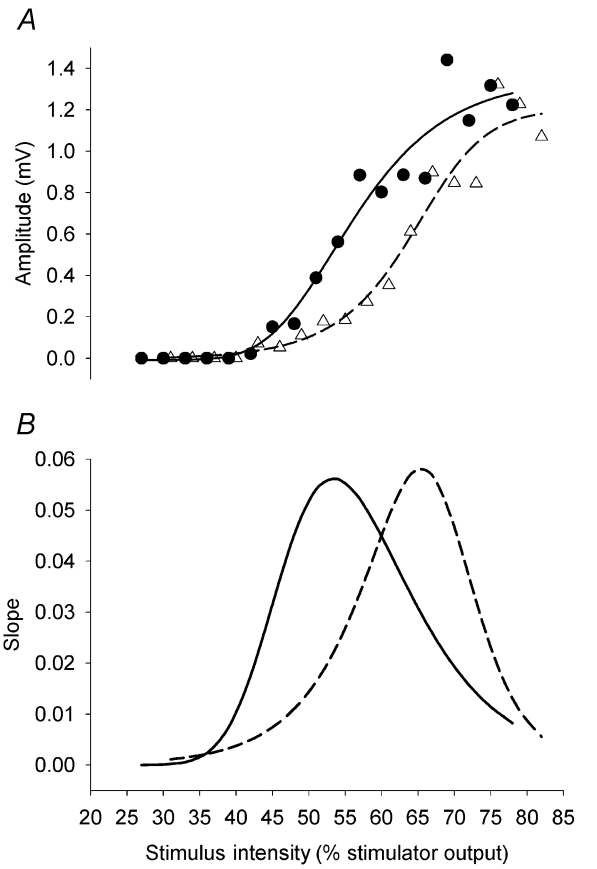

Figure 2. Stimulus–response relationships for resting FDI in two male subjects aged 29 years (●) and 46 years (△)

A, stimulus–response curves fitted with 5-parameter sigmoids. Note that the measured threshold to TMS and the MEP_{max} are similar in both, but that the curve for the older subject (dashed line) is shifted to the right. *B*, the slopes of the stimulus–response curves at each stimulus intensity, as derived from the fitted curves shown in *A*. The maximal slope is similar in both subjects but occurred at a lower stimulus intensity in the younger subject (continuous line).

with age in the stimulator intensity (both absolute and relative to resting threshold) above resting threshold at which the maximal rate of slope increase occurred (Age, $P = 0.003$, $n = 42$). The amplitude of MEP_{max} in older subjects was similar to that in the younger subjects, but required a higher stimulus intensity. The range of stimulus intensities over which MEPs were evoked (i.e. the stimulator intensity at MEP_{max} minus the stimulator intensity at which resting threshold occurred [% stimulator output]) was also greater in older subjects (Age, $P = 0.03$, $n = 42$).

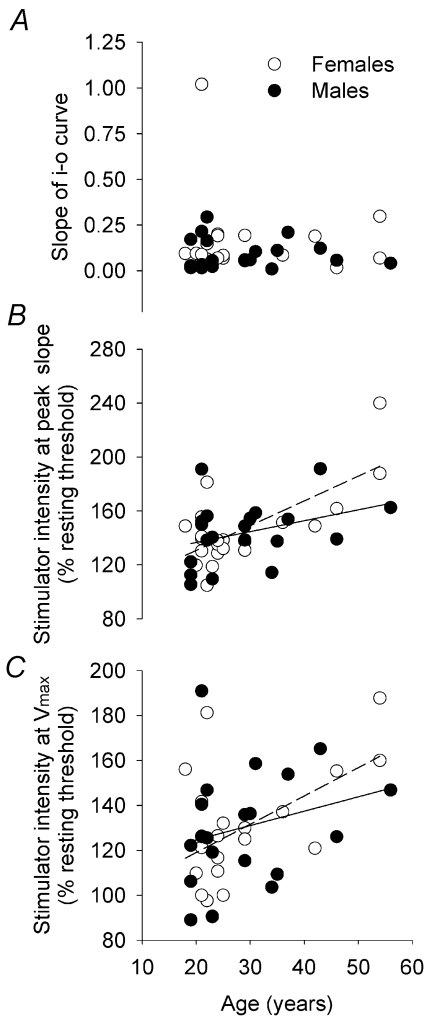

Figure 3. Stimulus–response characteristics: group data

A, the data points show the slope of individual curves. The slope of the input–output (i–o) curve was not affected by subject age or sex. *B*, however, the stimulus intensity (relative to resting threshold) at which the peak slope was attained increased with subject age (Age, $P \leqslant 0.0001$, $n = 42$). This was more evident in females (dashed line, $P = 0.002$, $n = 22$) than in males (continuous line, not significant (NS), $n = 20$) *C*, the maximum rate of slope change (V_{max}) attained was not altered by age. However, the stimulus intensity (relative to resting threshold) at which the maximum rate of slope increase was attained increased with age (Age, $P = 0.008$, $n = 42$). This was more evident in females (dashed line, $P = 0.01$, $n = 22$) than in males (continuous line, NS, $n = 20$). All data are individual subject responses.

The amplitude of MEP_{max} did not depend on either age or sex (Fig. 4*A*). Females (17.5 ± 4.7 mV) had larger M-waves than males (12.7 ± 3.5 mV) (Sex, $P = 0.04$, $n = 16$) and there was a trend (not significant) for M-wave amplitude to decrease with increasing subject age in both genders (Fig. 4*C*).

In order to determine whether the proportion of FDI muscle fibres activated by TMS changed with age, MEP_{max} was expressed as a proportion of the maximal M-wave amplitude. When all of the available data were included ($n = 16$), the weak trend for an age-related increase in MEP_{max}/M_{max} was not significant (Age, $P = 0.07$) (Fig. 4*D*). However, Fig. 4*D* shows that, when the female data were

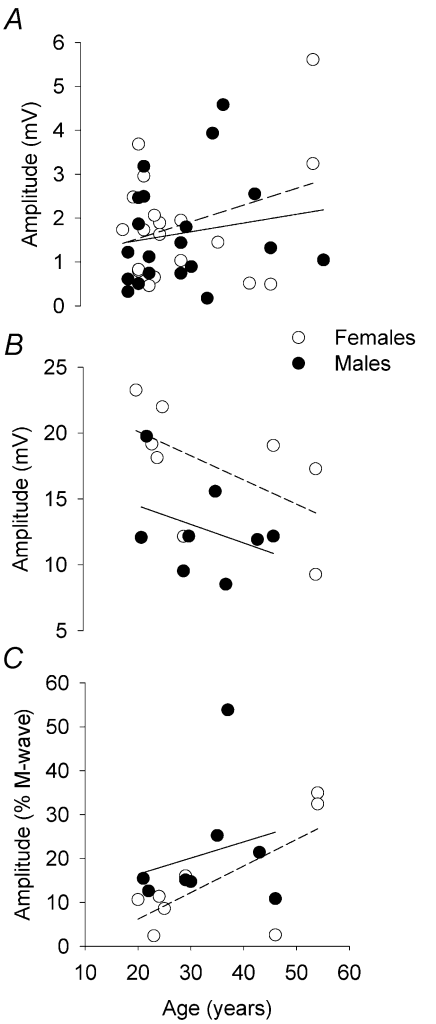

Figure 4. Relationships of MEP_{max} and M-wave to age and sex for 42 subjects

A, the measured MEP_{max} was not affected by age or sex. *B*, M-wave amplitude tended to decrease with age although this was not significant in the 18 subjects tested. *C*, the MEP_{max} expressed as a percentage of maximal M-wave amplitude tended to increase with age, but this was significant only in females (dashed line, Pearson's $r = 0.74$, $P = 0.05$, $n = 8$). Dashed correlation lines illustrate females and continuous lines are males. $n = 42$ for MEP data (i.e. *A*). $n = 16$ for M-wave data (i.e. *B* and *C*).

considered alone, this positive relationship was significant ($P = 0.05$, $n = 8$). There was no relationship between the resting threshold and MEP_{max}, nor was there any influence of M-wave size on MEP_{max}.

Effect of sex

Covariate analyses using age and sex showed that, while age influenced a number of MEP response characteristics, there were no differences due to sex, and age did not interact with sex to produce the changes. The estimate of the effect size of age and sex indicated a negligible effect of sex ($\eta_p^2 < 3\%$) but a large effect of age ($\eta_p^2 \geqslant 25\%$) on the stimulus–response profiles. The estimate of effect size gives a partial eta-squared value (η_p^2) each for the effect of sex and of age and therefore describes the proportion of total variance in a stimulus–response characteristic attributable to either age or sex. However, when males and females were considered separately, the age-related changes were evident only in females. Attempts to account for any outlier values in the regression failed to indicate a possible source for this difference. Therefore, it is not possible to determine from these data whether or not there is a difference in corticospinal responses between males and females with increasing age. However, for comparison, the regressions for each sex have been included in each of the figures, in addition to the overall change with age.

MEP and M-wave variability

To assess the trial-to-trial variability of the MEPs, the CV of MEP amplitude was computed for each run of ten consecutive MEPs at a given stimulus intensity, beginning with resting threshold. For two subjects, only the averaged MEP was recorded at each stimulus intensity, hence $n = 40$ for the CV data. The largest number of 3% steps (of stimulator output) above resting threshold in which all 40 subjects contributed data was eight, i.e. up to 21% of stimulator output above resting threshold. In an attempt to account for the diminishing subject numbers at higher stimulus intensities (i.e. due to subjects reaching MEP_{max} at less than 21% output above threshold), relationships affecting CV are described at two levels; firstly, where $n = 40$, i.e. at 21% of stimulator intensity above resting threshold; and secondly, at the highest stimulus intensity above threshold at which the relationship was still evident, regardless of subject number.

MEP CV decreased with increasing stimulus intensity (stimulus intensity 0–21% above threshold, $P \leqslant 0.0001$, $n = 40$; stimulus intensity 0–48% above threshold, $P = 0.005$, $n = 4$; Fig. 5). MEP CV tended to be highest at or near resting threshold, then decline rapidly with increasing stimulus intensity. MEP CV tended to reach a plateau (i.e. it neither increased or decreased with further increases in stimulus intensity) at approximately 20–25% of stimulus output above resting threshold (Fig. 5). However, even when there were no further increases in MEP amplitude with increases in stimulus intensity, MEP CV never reached zero. Figure 5 shows that MEP CV only fell to a mean of approximately 0.3 when the MEP amplitude was maximal.

The higher the subject's resting threshold, the greater the CV at any given stimulus intensity (stimulus intensity × resting threshold, 0–21%, $P = 0.035$, $n = 40$; stimulus

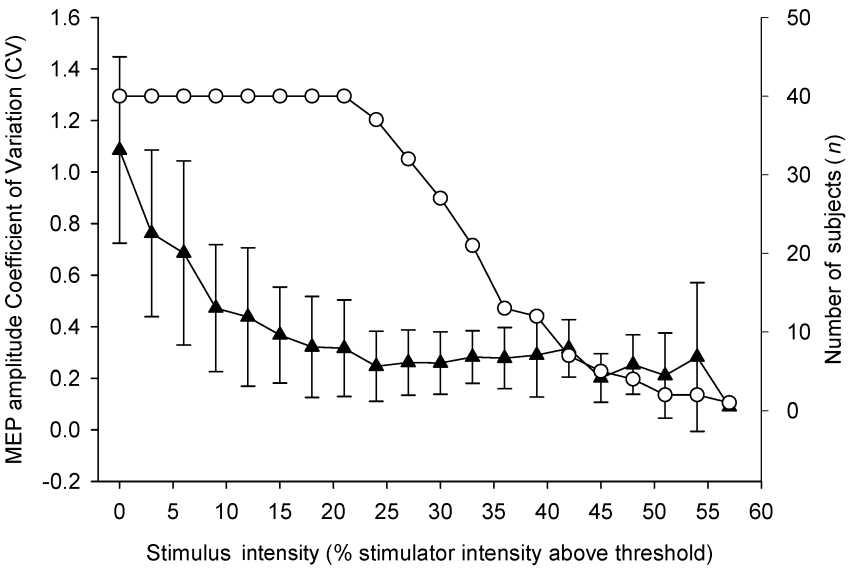

Figure 5. The trial-to-trial variability of MEP amplitude for 40 subjects

The data shown by filled triangles are the group mean CVs ± S.D. normalized to resting threshold (left axis). Resting threshold is shown as a stimulus intensity of zero, after which stimulus intensity was increased in 3% steps. The open circles show the number of subjects for whom data were obtained at each stimulus intensity (right axis). The CV at each stimulus was greatest at resting threshold, but decreased with subsequent increases in stimulus intensity, until reaching a plateau.

intensity × resting threshold, 0–48 %, $P \leqslant 0.0001$, $n = 4$). While there was no correlation between increasing subject age and resting threshold, there was a significant covariate interaction between age, resting threshold and MEP CV at a given stimulus intensity (stimulus intensity × resting threshold × age, 0–21 %, $P \leqslant 0.0001$, $n = 40$; stimulus intensity × resting threshold × age, 0–42 %, $P = 0.05$, $n = 7$). Older subjects tended to have higher resting thresholds and this was associated with increased variability of MEP amplitude, as stimulus intensity increased. Overall, MEP CV fell markedly as stimulus intensity increased above threshold. However, in older subjects, this intensity-related reduction in MEP CV was not as large or as rapid as in younger subjects. In addition, a higher resting threshold was associated with a 'slower' decline in MEP CV as stimulus intensity was increased; higher resting threshold was associated with increased subject age.

Subject sex alone did not exert an effect, but interacted with age and resting threshold to modulate MEP CV (stimulus intensity × age × resting threshold × sex; 0–21 % above threshold; $P = 0.011$, $n = 40$)(stimulus intensity × age × resting threshold × sex; 0–39 % above threshold; $P = 0.009$, $n = 21$). Females tended to have higher MEP CVs than males, but age and resting threshold were much stronger modulators of MEP CV than sex.

The mean coefficient of variation for M-waves (0.009) was significantly smaller than that for MEP_{max} (0.28) (ANOVA, $P \leqslant 0.0001$, $n = 16$). Neither M-wave trial-to-trial variability nor the variability difference when compared with MEP_{max} was influenced by either sex or age. In addition, there was no relationship between the magnitude of M-wave variability and that of MEP_{max}.

DISCUSSION

This study reveals the existence of age-related changes in the corticospinal projection to the resting FDI in normal adult humans. While the resting threshold for activation of the corticospinal system and the amplitude of the MEP_{max} were unaffected, there were marked age-dependent changes in the input–output characteristics of the system, with greater stimulus intensities required to reach the same maximal motor output in the older subjects. While the variability of trial-to-trial MEP amplitudes decreased with increasing stimulus intensity, this variability was greater in older subjects and was influenced by resting threshold and, to a lesser extent, sex, with females tending to have greater MEP variability than males of the same age. Several previous studies have shown that cortical excitability in females is modulated by changing ovarian steroid levels during the various stages of the menstrual cycle, which may account for this increased variability (Smith *et al.* 1999, 2002; Wassermann 2002).

For a threshold MEP to be evoked, first the cortico-motoneurones and then the spinal motoneurones must be brought to their respective firing thresholds. It is clear that tonic inputs to the motor cortex from other areas (e.g. thalamus, cortico-cortical projections) and tonic peripheral afferent signals (Ridding *et al.* 2000, 2001) are likely to influence the resting excitability of cortico-motoneurones. The excitability of spinal motoneurones is also subject to the influence of diverse, tonic inputs. The absence of an age-dependent effect on recruitment threshold suggests that these various factors do not change significantly with age.

The main effects of increasing age on corticospinal stimulus–response characteristics were a reduced rate of increase in MEP amplitude between resting threshold and approximately 50 % of MEP_{max} (e.g. Fig. 2*B*), and an increased variability in the amplitude of consecutive MEPs evoked at a given stimulus intensity. Although greater stimulus intensities above resting threshold were necessary to evoke them, the same MEP_{max} and maximum slope of increase were found in older subjects as in younger subjects.

Low-intensity TMS excites corticospinal neurones indirectly via interneurones that activate a series of indirect or 'I-waves' in the descending volley: higher intensities of stimulation evoke not only I-waves but also a D-wave that arises from direct activation of the corticospinal tract (Day *et al.* 1989; Rothwell *et al.* 1991; Dilazzaro *et al.* 1998). At TMS intensities near resting motor threshold, therefore, the temporal summation of I-waves at motoneuronal cell bodies must be an important determinant of MEP size (Mills 1991; Rothwell *et al.* 1991); that is, MEP amplitude is highly dependent on the level of excitability of interneurones in the motor cortex. At higher TMS intensities, the larger, highly synchronous D-wave volley probably has more influence on MEP amplitude than I-waves.

Neither motor threshold nor the amplitude of the MEP_{max} changed with subject age, but the amplitude of the MEPs increased more slowly in the older subjects as the TMS intensity increased, particularly between threshold and approximately 50 % MEP_{max}. The absence of change in threshold suggests that the threshold for I-wave production is not altered with increasing age. What, then, underlies the slower rate of increase of MEP amplitude at low-to-moderate stimulus intensities in the older subjects? There are two possible reasons for this phenomenon. Firstly, these lower stimulus intensities might result in fewer spinal motoneurones being activated synchronously in the older subjects. Alternatively, TMS might activate the same number of motoneurones but in a less synchronous manner, which would lead to phase cancellation of the action potentials of individual motor units and hence a

smaller peak-to-peak MEP amplitude (cf. Magistris *et al.* 1998, 1999).

Consider first the mechanisms that could give rise to activation of fewer motoneurones by low-to-intermediate TMS intensities. It is possible that the descending I-wave volley is relatively less synchronous at the lower TMS intensities in the older subjects, which would result in fewer motoneurones being recruited at a given TMS intensity. Higher TMS intensities would still activate a strong D-wave volley and perhaps also increase the synchrony of the I-waves in the descending volley. This would discharge the same overall proportion of motoneurones as in the younger subjects, giving rise to the same amplitude MEP_{max}.

Alternatively, the descending volley could be the same in the older subjects, but the pattern of recruitment of motoneurones could be different. The smaller motoneurones would continue to be activated by a similar descending volley at threshold, but the threshold for discharging the motoneurones of intermediate size could be greater. Fewer motoneurones would then be activated by the same descending volley at intermediate TMS intensities, resulting in smaller MEPs. However, a supra-maximal volley could still discharge the same overall proportion of motoneurones as in the younger subjects, giving rise to a similar amplitude MEP_{max}. This change in recruitment pattern could, for example, result from changes in the synaptic density on spinal motoneurones, which is said to decrease with age (Eisen *et al.* 1996). However, for this explanation to hold, the synaptic density on small motoneurones would need to be retained to explain the lack of change in threshold.

Smaller MEPs would also be evoked by low-to-intermediate TMS intensities in older subjects if the same number of motoneurones was activated, but in a less synchronous manner than in the younger subjects. If the activation of the various motoneurones innervating a given muscle is dispersed over several milliseconds, the amplitude of the MEP will be diminished as the result of phase cancellation of the action potentials of the motor units as they become activated (Magistris *et al.* 1998, 1999). Hence the smaller MEPs in the older subjects could be the result of increased temporal dispersion of the corticospinal volley at low-to-intermediate TMS intensities, presumably as the result of more temporally dispersed I-waves. As before, stronger TMS could overcome this effect by activating a strong D-wave volley and perhaps also increasing the synchrony of the I-waves to elicit the same MEP_{max}.

The distinct changes in MEP variability with increasing subject age provide some support for both the temporal dispersion and the fewer-motoneurones theories, without eliminating one or the other. Older subjects had significantly more variability of MEP amplitude over ten consecutive MEPs. The origin of trial-to-trial fluctuation in MEP amplitude at a given stimulus intensity is unknown but is probably caused by rapidly fluctuating changes in cortical and spinal excitability (Kiers *et al.* 1993; Ellaway *et al.* 1998; Funase *et al.* 1999). An increased moment-to-moment fluctuation in cortical excitability with age is one possible explanation for the age-related increase in the trial-to-trial variability in MEP amplitude observed. It could also explain the age-related interaction between resting threshold and MEP variability. The commonly used working definition of resting threshold, also used in the present study, is a minimum of five out of ten consecutive MEPs with a minimum amplitude of 50 μV. Since MEP CV is largest at or around resting threshold (Fig. 5), it is a statistical probability that older subjects tend to require larger stimulus intensities to satisfy the definition. However, there was no direct relationship between age and increasing threshold. Equally, the age-related increase in MEP variability could be attributable to fewer motoneurones within the target pool, for the same reasons as outlined earlier.

Figure 5 shows that, regardless of age, the coefficient of variation of the MEP response decreased as the stimulus intensity increased. Furthermore, even at high TMS intensities where MEP amplitude was saturated, and where one might expect that every corticospinal neurone and motoneurone that could be activated was activated, the variability rarely fell below about 0.3. The variation in response from trial to trial is the result of fluctuations of the excitability of individual neurones from one moment to the next. At MEP threshold, there must be a small number of corticospinal neurones and motoneurones whose membrane potentials are near their firing threshold at any instant. Most of these will fire when a weak excitatory input is delivered. However, a variable number do not. Some tonically active corticospinal motoneurones may fail to discharge either because, having just fired, they are still hyperpolarized. Alternatively, their membrane potentials may be hyperpolarized because they are subject to net inhibition at the time of the stimulus. M-waves are not subject to these refractory effects on motoneurones and this is reflected in their significantly smaller coefficients of variation. The spinal motoneurones are also subject to fluctuating levels of inhibitory and excitatory inputs which may cause them to be hyperpolarized at the time that the descending volley arrives and hence not discharge. However, they are not discharging tonically in these experiments and for this reason cannot be refractory.

The same arguments prevail at TMS intensities up to maximal. That is, a variable number of corticospinal and motor neurones will not discharge even with a powerful input. The observation that the CV decreases to a plateau

at higher stimulus intensities suggests that the proportion of non-discharged neurones remains relatively stable, and that the CV (standard deviation expressed as a proportion of the mean) is less primarily because the mean amplitudes of the MEPs are larger.

There is evidence from both histological and electrophysiological studies for age-related changes in cortical and corticospinal cytoarchitecture in humans, beginning as early as the third decade. Approximately 30 % of the corticospinal tract fibres originate in the primary motor cortex (Brodman's area 4), 30 % from the premotor areas (area 6) and the remainder from the somatosensory cortex (areas 1, 2 and 3) (Jane *et al.* 1967; Kandel *et al.* 1991; Galea & Darian Smith, 1994). Ultrastructural analyses of age-related changes in synaptic architecture have shown that, in a normal human population aged 45–84 years, increasing age is associated with a decrease in synapse number and an increase in the length of the postsynaptic contact zone in the area 4/primary motor cortex, but not area 3/layer 1 of the somatosensory cortex (Adams, 1987). Electrophysiological studies have reported an age-dependent, linear decline in the amplitude of the excitatory postsynaptic potential evoked by TMS in extensor digitorum communis motoneurones in 42 normal subjects aged between 24 and 83 years (Eisen *et al.* 1996). They estimated that approximately 35 % of cortico-motoneurones are either lost or non-functional in normal humans by the age of 50 years. While this fits with the increased stimulus intensities required to produce a given amplitude MEP in older subjects, it might also be expected that it would be associated with a higher resting motor threshold. However, evidence from studies in patients with early-stage amyotrophic lateral sclerosis suggests that the initial loss of cortico-motoneurones reduces the effectiveness of I-wave summation at the anterior horn cell without a concomitant increase in corticomotor threshold, although threshold eventually increases as the disease progresses (Eisen & Swash, 2001). This explanation again requires preferential sparing of the corticospinal projection onto small motoneurones to explain the lack of change in resting threshold.

Finally, the effect of age on the motoneurones themselves should be considered. There is a large literature that indicates that the loss of human muscle mass and strength with age is due primarily to the loss of α-motoneurones and the subsequent denervation of muscle fibres (reviewed in Doherty *et al.* 1993). However, these changes are generally not evident until the seventh and eighth decades. For example, Tomlinson & Irving (1977) studied the lumbosacral spinal cords of 47 subjects post mortem, aged 13 to 95 years, and found no evidence of reduced motoneurone numbers below the age of 60 years. Therefore it is unlikely that loss of α-motoneurones is a factor in the current study where the oldest subject was 55 years old.

In summary, there are age-related changes in the strength of the corticospinal projection to FDI in normal human adults, that are manifest as a shift to the right of the cortically-evoked stimulus–response curve without concomitant changes in resting motor threshold or MEP_{max}. These changes are likely to be the result of changes in the motor cortex with increasing age, namely, reduced synchronization of I-waves in the descending volley, or the loss of cortico-motoneurones. In terms of current methodologies for TMS studies, these findings suggest that there are limitations inherent in selecting stimulating intensities relative to resting threshold, unless subject groups homogenous for age are used.

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