

Activity-dependent depression of the recurrent discharge of human motoneurons after maximal voluntary contractions

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Key points

- During fatiguing contractions, the output of the motoneurone pool decreases despite maximal voluntary drive. The precise mechanism for this reduction is unknown.
- To assess whether changes in the intrinsic behaviour of motoneurons due to their repetitive activity during maximal voluntary contractions are responsible for the reduction of motoneurone output, we measured the recurrent motoneurone discharge produced antidromically by supramaximal stimulation of peripheral nerve. These recurrent responses are known as F-waves when measured in the EMG.
- Maximal voluntary contractions strongly depressed the probability of occurrence of F-waves and their area when measured at rest after the contractions. The strength and time course of the depression increased with the duration of the contraction for the abductor digiti minimi muscle. Moreover, the observed depression of F-waves could not be explained by altered excitability of the peripheral motor axons.
- Hence, we propose that the depression in F-waves is caused by activity-dependent changes at the soma or the initial segment and that this may contribute to central fatigue.

Abstract Despite maximal voluntary effort, the output of human motoneurone pools diminishes during fatigue. To assess motoneurone behaviour, we measured recurrent discharges evoked antidromically by supramaximal nerve stimulation after isometric maximal voluntary contractions (MVCs). They were measured as F-waves in the electromyographic activity (EMG). Supramaximal stimuli to the common peroneal and ulnar nerves evoked F-waves at rest before and after MVCs in tibialis anterior (TA) and abductor digiti minimi (ADM), respectively. F-waves were depressed immediately after a sustained MVC. For TA, the size and time course of depression of the F-wave area ($26 \pm 13\%$; mean \pm SD; $P = 0.007$) and persistence ($\sim 20\%$) were similar after a 10-s or 1-min MVC. For ADM, the decline in F-wave area ($39.8 \pm 19.6\%$; $P < 0.01$) was similar after the two contractions but the decline in persistence (probability of occurrence) of the F-wave differed ($14.6 \pm 10.5\%$ and $32.5 \pm 17.1\%$ after 10-s and 1-min MVCs respectively). Comparison of a very long (2-min) with a very short (2-s) MVC in ADM showed that the depression of F-wave area, as well as persistence, was greater after the longer contraction. This suggests, at least for ADM, that the depression is related to the duration of voluntary activity and that the decrease in F-waves could contribute to central fatigue. To examine whether changes in motor axon excitability caused the depression, we measured compound muscle action potentials (M-waves) to submaximal stimulation of the ulnar nerve after a 2-s and 2-min MVC. Submaximal M-waves were not depressed after a 2-s MVC. They were depressed by a 2-min MVC, but the time course of depression of the F- and M-waves differed. Thus, depression of F-waves does not simply

reflect reduced excitability of peripheral motor axons. Hence, we propose that activity-dependent changes at the soma or the initial segment depress the recurrent discharge of human motoneurons and that this may contribute to central fatigue.

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Abbreviations ADM, abductor digit minimi; CMEP, cervicomedullary motor evoked potential; EMG, electromyographic activity; Mmax, maximal compound muscle action potential; MEP, motor evoked potential; MVC, maximal voluntary contraction; TA, tibialis anterior.

Introduction

During a sustained isometric maximal voluntary contraction (MVC), the firing rates of motoneurons decline progressively (e.g. Bigland-Ritchie *et al.* 1983) and 'central' fatigue develops (e.g. Gandevia, 2001). In addition, the response to stimulation at the cervicomedullary junction (cervicomedullary motor evoked potential, CMEP), which largely depends on the excitability of the spinal motoneurons, also declines when a maximal voluntary contraction is sustained for more than ~1 min (Butler *et al.* 2003; Martin *et al.* 2006). In more recent studies, further evidence of impaired 'excitability' of spinal motoneurons was derived from changes in CMEPs during transient disfacilitation of the motoneurone pool by a prior transcranial magnetic stimulus to the motor cortex. During this transient disfacilitation, the CMEP was abolished after ~20 s of MVC (McNeil *et al.* 2009, 2011). These changes in motoneurone excitability during an MVC do not appear to be due to altered input from group Ia (McNeil *et al.* 2011) or group III and IV muscle afferents (Martin *et al.* 2006). By exclusion, it has been proposed that they reflect changes in the intrinsic properties of the motoneurone. This would fit with some observations in animal preparations. When a constant current is injected into motoneurons, they fire initially at a high rate but slow with time (e.g. Kernell & Monster, 1982; Sawczuk *et al.* 1997). Such spike frequency adaptation may occur with repetitive firing of human motoneurons.

Various non-invasive techniques have been employed to assess the 'excitability' of motoneurons in humans. These include H-reflexes, CMEPs and the recurrent discharge of motoneurons (F-wave). Although H-reflexes can reveal changes in motoneurone behaviour, the size of the reflex response is greatly influenced by pre-synaptic inhibition of the Ia terminal (e.g. Hultborn *et al.* 1987; Meunier & Pierrot-Deseilligny, 1998) and activity-dependent changes in the afferent volleys (Burke & Gandevia, 1999). Thus, changes in H-reflex size do not necessarily reflect changes in the excitability of motoneurons. Unlike the H-reflex, the CMEP is not influenced by classical presynaptic inhibition (e.g. Nielsen

& Petersen, 1994; Jackson *et al.* 2006), but it is altered by pre-motoneuronal changes presumably at the cortico-motoneuronal synapse (Gandevia *et al.* 1999; Petersen *et al.* 2003). On the other hand, F-waves are produced by recurrent discharges of motoneurons following antidromic activation by supramaximal stimulation of a peripheral nerve. Studies on cat lumbosacral motoneurons indicate that only stimulated motoneurons discharge a recurrent volley (Renshaw, 1941; Lloyd, 1943) and that the site of axonal re-excitation can be the initial segment (Coombs *et al.* 1957a) or first node of Ranvier (Gogan *et al.* 1984). Thus F-waves should not depend on pre-motoneuronal factors but depend solely on the behaviour of motoneurons. The behaviour of F-waves has been used to track motoneurone excitability in clinical settings. F-waves increase in size and probability of occurrence (persistence) with voluntary contraction of the target muscle (McComas *et al.* 1970; Fox & Hitchcock, 1987; Espiritu *et al.* 2003; Hara *et al.* 2010) and they are larger and easier to elicit in spasticity (e.g. Eisen & Odusote, 1979; Bischoff *et al.* 1992; Milanov, 1994; Argyriou *et al.* 2010). Recent work has highlighted the importance of the axon initial segment (Clark *et al.* 2009; Grubb & Burrone, 2010; Wimmer *et al.* 2010; Debanne *et al.* 2011) and first node of Ranvier (Kole, 2011) in controlling neuronal firing properties over short and long time scales.

Prior studies give some evidence for a reduction in motoneurone excitability after repetitive firing. The mean area of F-waves decreased in the first dorsal interosseus muscle after a 10-s MVC (Giesebrecht *et al.* 2011) and in abductor digit minimi after a 1-min MVC (Rossi *et al.* 2010). However, as only one duration of contraction was used in each study, it is not clear whether the depth and duration of the depression depends on the duration of activity and thus may reflect a motoneuronal mechanism which could contribute to human muscle fatigue. Rossi and colleagues (2010) suggested that the post-contraction reduction in F-wave area and persistence and depressions of H-reflexes and responses to transcranial magnetic stimulation are all associated with the known activity-dependent hyperpolarisation of the peripheral motor axon (e.g. Schoepfle & Katholi, 1973; Bostock & Grafe, 1985; Vagg *et al.* 1998).

The current study was designed to determine whether the depression of F-waves after MVCs is related to the duration of motoneuronal activity. For this purpose, we examined F-waves during relaxation after different duration MVCs in an intrinsic hand muscle (abductor digit minimi, ADM) and the major dorsiflexor of the ankle (tibialis anterior, TA). We hypothesised that F-waves would show activity-dependent changes so that a longer MVC would induce a deeper and longer depression of F-waves. Furthermore, to examine whether activity-dependent changes in motor axons contribute to the depression in F-waves, we also investigated compound muscle action potentials (M-waves) produced by different intensities of ulnar nerve stimulation.

Methods

In two studies, F-waves were examined in a lower leg (tibialis anterior; study 1) and an intrinsic hand muscle (abductor digit minimi; study 2) after 10-s and 1-min maximal voluntary contractions (MVCs). Eight healthy subjects (4 male and 4 female; 31 ± 7 years; mean \pm SD) participated in the study. Five of these subjects plus a further three (3 male and 5 female; 34 ± 6 years) participated in the third study which examined the F-waves in abductor digit minimi before and after a very short (2-s) and a very long (2-min) MVC. Three subjects from study 3 plus a further five participated in an additional experiment which examined M-waves after a 2-s and a 2-min MVC. Each subject gave written informed consent to the experimental procedures, and the local human research ethics committee approved the study. The study was conducted according to the *Declaration of Helsinki*.

Experimental set-up

In study 1, subjects sat upright in a customised chair with knee and ankle flexed at 90 deg. The right foot was strapped to an isometric myograph to record dorsiflexor torque at the ankle joint (Todd *et al.* 2004). In studies 2, 3 and 4, subjects sat in an adjustable chair with the right forearm and fingers rested on a table and the elbow flexed in a comfortable position. The forearm was pronated and secured by straps. The thumb rested in a midflexed position and the second to fourth fingers were secured by a strap. The little finger was placed firmly into a custom-designed finger splint with an axis of rotation about the metacarpophalangeal joint in a horizontal plane. The splint was connected to a force transducer to measure isometric finger abduction force. Surface EMG was recorded from TA and ADM using Ag/AgCl disk electrodes (6 mm in diameter) filled with conducting gel. The cathode was placed over the motor-point of each

muscle and the anode 2 cm distal to the motor-point. The EMG signals were recorded in two ways. The EMG signals were amplified ($\times 1000$), band-pass filtered (200–1000 Hz; 2-pole Bessel filter; CED 1902 amplifiers, Cambridge Electronic Design, Cambridge, UK) and sampled at 5 kHz. The high-pass cutoff was set at 200 Hz so that the tail end of the maximal M-wave (M_{\max}) had returned to baseline by the start of the F-waves (filtered F-wave; Fig. 1B). The EMG signals were also amplified ($\times 300$), band-pass filtered (10–1000 Hz; 2-pole Butterworth filter; CED 1902 amplifiers) and sampled at 2 kHz (Fig. 1A). Force data were sampled at 1000 Hz. All signals were stored on a computer (CED 1401 Plus) and Spike2 software (v. 6.13; Cambridge Electronic Design).

Stimulation

Common peroneal nerve stimulation. The common peroneal nerve was stimulated to evoke F-waves at rest in TA. The best location for the cathode was identified by stimulation with a hand-held electrode near the neck of the fibula. Single electrical stimuli (100 μ s duration) were delivered by a Digitimer DS7AH constant current stimulator (Digitimer, Welwyn Garden City, UK) through a custom-designed bar electrode (interelectrode distance 4 cm) with the cathode over the best site and the anode anterior. With the subject at rest, the stimulus intensity was gradually increased until the compound muscle action potential (M-wave) failed to increase in peak-to-peak amplitude despite an increase in current. To maintain supramaximal stimulation despite any effect of activity-induced hyperpolarisation of the motor axons during a 10-s or 1-min MVC, the intensity of stimulation was set at 150% of the intensity that evoked M_{\max} (30–105 mA).

Ulnar nerve stimulation. F-waves were evoked in ADM at rest by electrical stimuli (100 μ s duration) applied to the ulnar nerve. A bar electrode (interelectrode distance 4 cm) was positioned over the ulnar nerve on the ulnar side of the forearm with the cathode just proximal to the wrist joint. The size of M_{\max} was determined for each subject and the intensity of stimulation was set at 150% of the intensity required to produce a maximal M-wave (45–70 mA). For study 4 (see below), three separate stimulators were used and were set to evoke M-waves with an amplitude of 100%, 50% and 25% of M_{\max} .

Protocol

Study 1: F-waves in TA after a 10-s and a 1-min MVC. We examined F-waves in TA before and after a 10-s and a 1-min maximal voluntary contraction (MVC). The same subjects attended on two days. On the first visit,

subjects performed a 10-s MVC and on the second visit, a 1-min MVC. Two control sets of F-waves, separated by 1 min, were collected prior to the MVC (10-s or 1-min MVC). After another 1-min break, subjects performed an isometric MVC of the ankle dorsiflexors. During the maximal contraction, subjects received visual feedback of their performance and were verbally encouraged. Subjects relaxed immediately after the MVC, and six post-MVC sets of F-waves, each separated by 30 s rest, were collected. For each set, subjects were given 30 supramaximal stimuli (150% Mmax) delivered at 0.5 Hz to evoke F-waves with the muscle relaxed.

Study 2: F-waves in ADM after a 10-s and a 1-min MVC.

In study 2, we repeated the same protocol to examine whether similar changes occur in F-waves in ADM after a 10-s and a 1-min MVC. The same subjects as in study 1 ($n = 8$) attended on two more days. F-waves were evoked in ADM at rest by supramaximal electrical stimuli (150% Mmax) to the ulnar nerve given in sets of 30 at 0.5 Hz. Two control sets prior to maximal abduction of the little finger and six post-MVC sets were collected for each subject.

Study 3: F-waves in ADM after a 2-s and a 2-min MVC.

Because a 10-s and a 1-min MVC revealed similar activity-dependent changes in F-waves (see Results), we further examined the F-waves in the ADM muscle after a very short (2-s) and a very long (2-min) MVC. Five subjects from studies 1 and 2 plus a further three attended on one day. Using the same protocol as study 2, F-waves were collected before and after a 2-s MVC of ADM. After a rest of 10 min, the protocol was repeated with a 2-min MVC.

Study 4: M-waves in ADM after a 2-s and 2-min MVC.

To examine whether activity-dependent changes in the excitability of motor axons contribute to the depression of F-waves, we investigated M-waves, the direct muscle response to electrical stimulation of the motor axons, in ADM after a very short (2-s) and a very long (2-min) MVC. Three subjects from study 3 plus a further five attended on one day. Electrical stimuli (100 μ s duration) at intensities set to evoke 100%, 50% and 25% Mmax were delivered in order at 2 s intervals over the ulnar nerve to evoke M-waves in ADM at rest before and after a sustained MVC. Using the same protocol as study 3, M-waves were collected in sets of 30 (10 of each size) before and after a 2-s MVC of ADM and then after a 10 min rest, before and after a 2-min MVC.

Data analysis and statistics

All measurements were made offline using Signal software (v. 3.05; Cambridge Electronic Design). To measure the area and peak-to-peak amplitude of F-waves, responses

to 30 supramaximal stimuli were overdrawn on the screen at high gain and the cursors were set at the beginning and the end of the responses that showed a clear deflection from the baseline. M-waves elicited by supra-maximal (studies 1, 2 and 3), maximal and submaximal stimuli (study 4; 100, 50 and 25% Mmax) were superimposed, and both the area and peak-to-peak amplitude were measured between cursors that encompassed the evoked responses. Root mean square EMG was calculated over 50 ms immediately before each stimulus. The area and peak-to-peak amplitude of F-waves measured from the heavily filtered EMG showed similar results and area is reported throughout the text. Because of activity-dependent changes in muscle fibre action potentials during maximal efforts, the area of each F-wave was normalised to the area of the corresponding Mmax (also heavily filtered) and then expressed as a percentage of mean control values (mean of control set 1 and 2) obtained prior to the MVC (Fig. 1C) (Taylor *et al.* 1999). For each subject, the mean area of the 30 F-waves in each set were used for statistical analysis. To illustrate in more detail the time course of changes, figures show the F-wave area averaged for each 10 consecutive stimuli (Espiritu *et al.* 2003). In addition, as F-waves are not seen after every supramaximal stimulus, we measured the number of F-waves which occurred with each set of 30 consecutive stimuli for both TA and ADM. This value was expressed as a percentage. This measure of the probability of the occurrence of F-waves is known as persistence. Assessment based on 30 trials can be considered sufficient (review Panayiotopoulos & Chroni, 1996). We identified an F-wave as present if a response with an appropriate latency (minimum of 20 ms and 30 ms for ADM and TA, respectively) had an amplitude $\geq 20 \mu$ V.

For studies 1, 2 and 3, F-wave area was analysed with two-way repeated measures analyses of variance (ANOVA) with factors of contraction duration and time. Whenever the ANOVA showed a significant main effect of time or contraction or a significant interaction, *post hoc* Student–Newman–Keuls tests were used to identify differences between the control and subsequent time points or between contractions. Because data for F-wave persistence were not normally distributed, we rank-transformed the data and performed a two-way parametric repeated measures ANOVA for comparison of contraction duration and time. A *post hoc* Student–Newman–Keuls test was used to identify differences between the control and subsequent time points or between contractions. For study 4, M-wave area was analysed using one-way repeated measures ANOVA to assess the main effects for time at the three stimulus intensities (100%, 50% and 25% Mmax). These were followed by a *post hoc* Dunnett's test. When the assumption of sphericity was violated, the *P* values were corrected using the Greenhouse–Geisser corrections.

Data are represented as means \pm SD in the text and means \pm SEM in the figures. Statistical significance was set at $P < 0.05$.

Results

Study 1: F-waves at rest after a 10-s and a 1-min MVC in TA

F-waves were evoked in the relaxed TA muscle before and after a 10-s and a 1-min MVC. Figure 2A shows

superimposed raw traces for a single subject for both durations of contraction. F-wave area decreased significantly with time ($F_{6,42} = 3.492$, $P = 0.007$) but the depression was similar after the two contractions ($F_{1,7} = 0.079$, $P = 0.787$), and there was no significant interaction ($F_{6,42} = 0.332$, $P = 0.916$) (Fig. 2B). *Post hoc* testing showed that the depression of $26 \pm 13\%$ of the initial control values was significant ($P = 0.007$) for the first minute after contraction. F-wave persistence was calculated for each set of 30 consecutive stimuli. Baseline F-wave persistence in TA was reproducible in the two

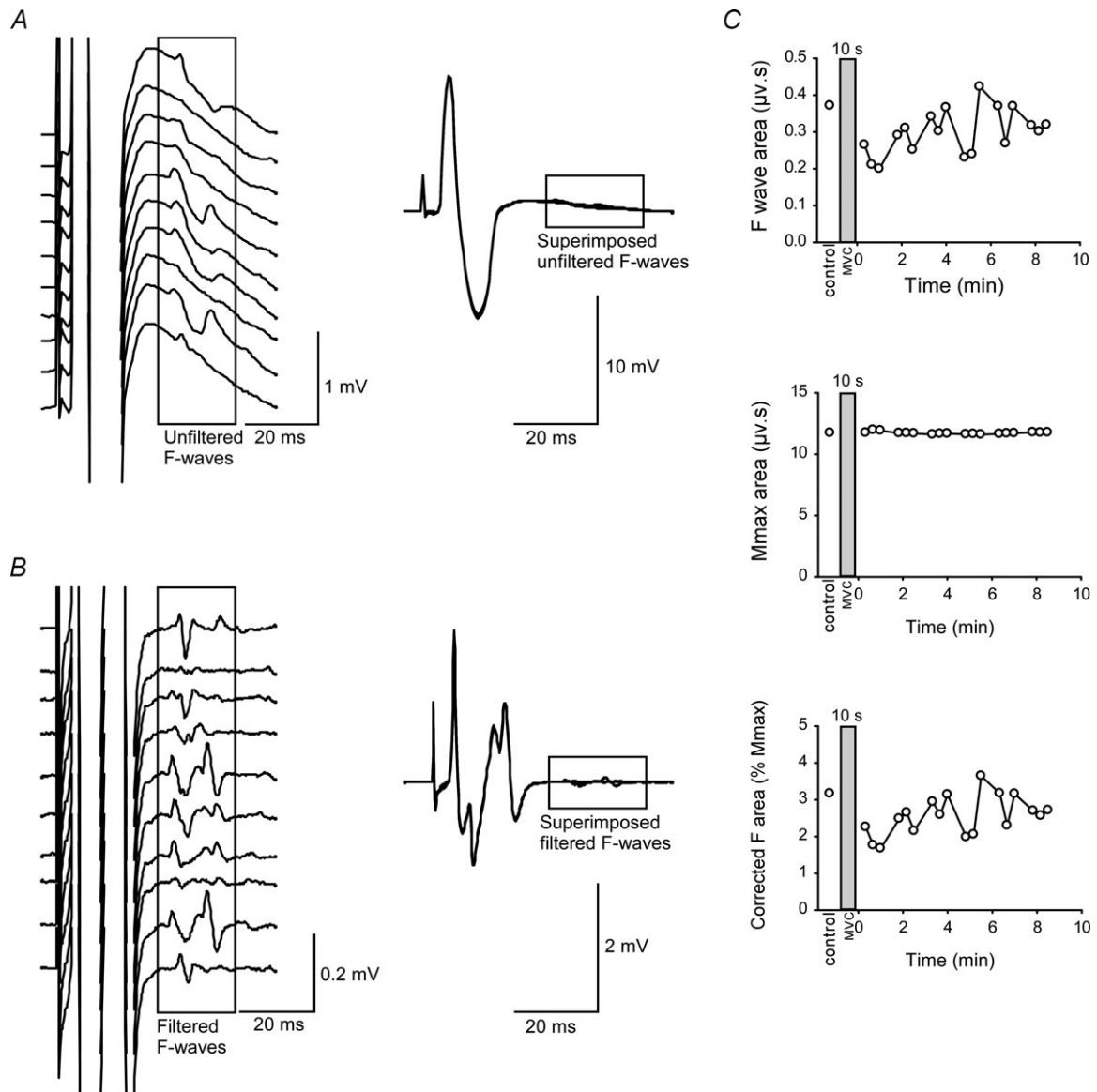


Figure 1. Measurement of F-waves
 A, left panel shows a raster of 10 consecutive F-waves recorded from ADM with filtering of 10–1000 Hz (unfiltered F-waves). Right panel shows the same 10 superimposed Mmax responses and F-waves. B, left panel shows raster of the same 10 consecutive F-waves heavily high-pass filtered (200–1000 Hz; filtered F-waves). Right panel shows filtered superimposed Mmax responses and F-waves. C, top panel shows absolute area of filtered F-waves before and after a 10-s MVC in one subject. Middle panel shows absolute area of filtered Mmax and bottom panel shows corrected area of the F-wave as a percentage of Mmax. The shaded rectangle denotes a sustained MVC.

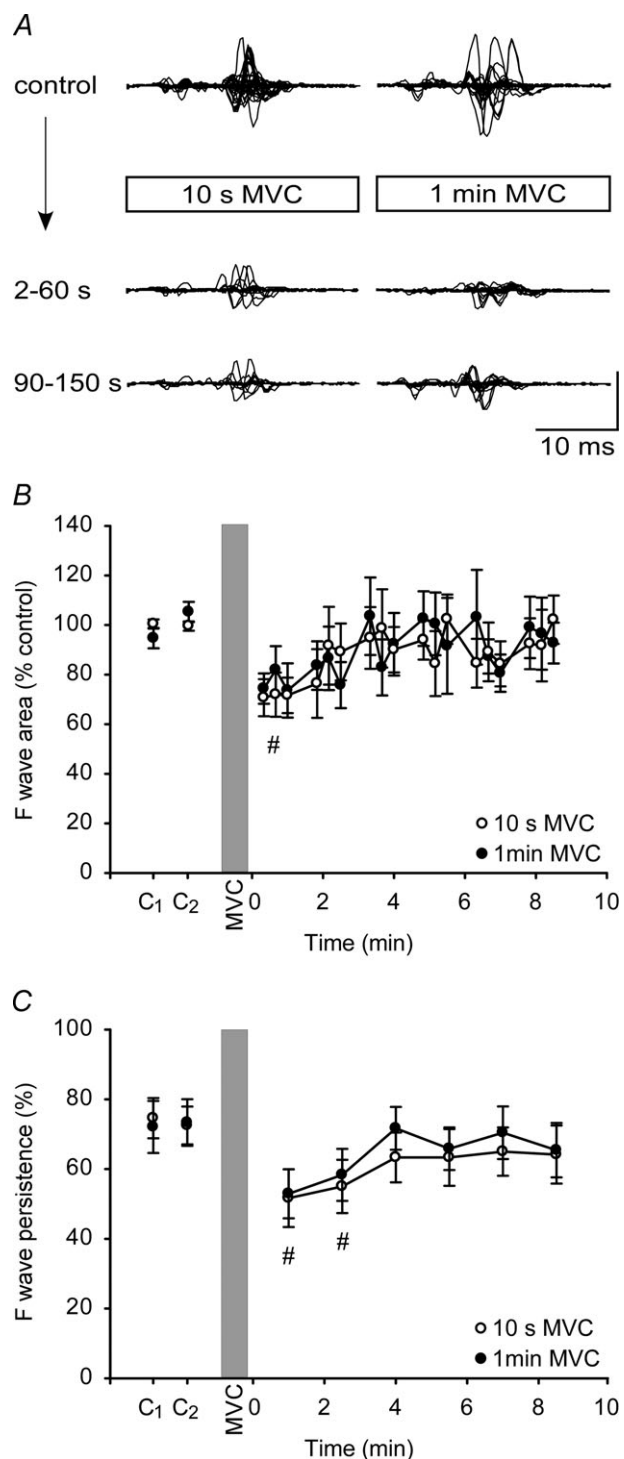


Figure 2. F-waves in tibialis anterior (TA) after MVCs of 10 s and 1 min duration

A, F-waves recorded from TA at rest before and after a 10-s (left) and a 1-min (right) sustained MVC for a single subject. In each panel, top traces represent one set of F-waves recorded before the MVC and are shown superimposed. Lower traces show superimposed responses for the first and second sets after the MVC. Each set consisted of 30 F-waves. Vertical calibration: 0.2 mV. B, group data ($n = 8$; means \pm SEM) showing changes in the area of F-waves after

sets of 30 stimuli delivered prior to each contraction, and on the two days (72.0–74.5%; Fig. 2C). F-wave persistence declined by $21.9 \pm 15.4\%$ and $19.8 \pm 21.8\%$ after a 10-s and a 1-min MVC, respectively (Fig. 2C). Two-way ANOVA showed a significant effect of time ($F_{6,42} = 5.44$, $P < 0.001$) but no effect for contraction ($F_{1,7} = 0.29$, $P = 0.61$) and no significant interaction ($F_{6,42} = 0.248$, $P = 0.96$) (Fig. 2C).

Study 2: F-waves at rest after a 10-s and a 1-min MVC in ADM

In study 2, we examined F-waves in ADM after a 10-s and a 1-min MVC to see whether F-waves showed similar changes in an intrinsic hand muscle after contraction. Superimposed F-waves before and after the MVCs from a typical subject are shown in Fig. 3A. As in study 1, significant depression of the F-wave occurred with time ($F_{6,42} = 9.826$, $P < 0.001$), but the depression was similar after a 10-s and a 1-min MVC ($F_{1,7} = 1.967$, $P = 0.204$) and there was no significant interaction ($F_{6,42} = 1.552$, $P = 0.185$) (Fig. 3B). The initial reduction in F-wave area was $39.8 \pm 19.6\%$ of the control value, and the depression lasted for ~ 6 min ($P < 0.01$) (Fig. 3B). F-wave persistence was high and reproducible in ADM (baseline persistence between 96.3 and 98.3% for each set of 30 stimuli; Fig. 3C). Two-way ANOVA showed a significant effect for contraction duration ($F_{1,7} = 9.91$, $P = 0.016$), and time ($F_{6,42} = 13.81$, $P < 0.001$), and an interaction ($F_{6,42} = 5.85$, $P < 0.001$). After a 10-s MVC, *post hoc* tests showed that F-wave persistence declined by $14.6 \pm 10.5\%$ and remained significantly decreased for 1 min compared to the mean baseline values ($P = 0.002$; Fig. 3C). After a 1-min MVC, persistence decreased by $32.5 \pm 17.1\%$ and remained reduced for 3 min ($P = 0.02$; Fig. 3C). Comparison between the contractions showed persistence was more depressed after the 1-min MVC than the 10-s MVC for the first 3 min after contraction (Student–Newman–Keuls; $P < 0.002$).

a 10-s (open circles) and a 1-min sustained MVC (filled circles). In this and subsequent figures, F-wave area was corrected to the respective Mmax response and then normalised to the mean value of the control sets. For illustration, each circle represents the average area of 10 consecutive F-waves from each subject. For statistical tests, F-waves were grouped into sets of 30. C, group data showing F-wave persistence in the relaxed muscle before and after a 10-s (open circle) and a 1-min (filled circle) MVC. The persistence was calculated from a set of 30 consecutive stimuli. The shaded vertical bar denotes the sustained MVC. There was an overall effect of time on F-wave area and persistence and the hash (#) indicates sets of F-waves for which values are significantly lower than the combined baseline values ($P < 0.05$; see Results)

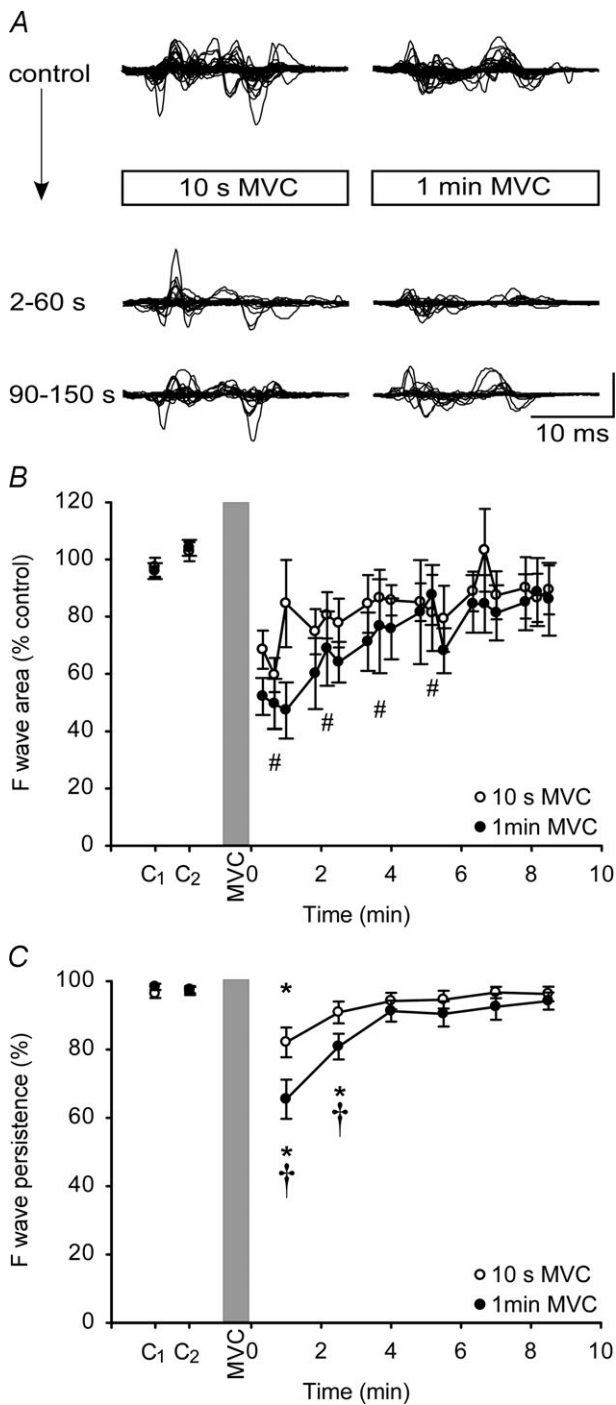


Figure 3. F-waves in abductor digit minimi (ADM) after MVCs of 10 s and 1 min duration
 A, F-waves recorded from ADM at rest before and after a 10-s (left) and a 1-min (right) sustained MVC for a single subject. In each panel, top traces represent F-waves recorded before the MVC and are shown superimposed. Lower traces show superimposed responses for the first and second sets after the MVC. Each set consisted of 30 F-waves. Vertical calibration: 0.2 mV. B, group data ($n = 8$; means \pm SEM) showing changes in the area of F-waves after a 10-s (open circles) and a 1-min sustained MVC (filled circles). For illustration, each circle represents the average area of 10 consecutive F-waves from each subject. For statistical tests, F-waves were

Study 3: F-waves at rest after a 2-s and a 2-min MVC in ADM

F-waves were examined during relaxation after a very short, 2-s MVC, and a very long, 2-min MVC. Depression of the area of the F-waves was more prolonged after the 2-min MVC as shown for a typical subject in Fig. 4A. Unlike studies 1 and 2, F-wave area showed a significant effect for contraction duration ($F_{1,7} = 12.1$, $P = 0.01$), and time ($F_{6,42} = 8.852$, $P < 0.001$), and an interaction ($F_{6,42} = 4.073$, $P = 0.003$). *Post hoc* tests showed that F-wave area compared to the control value was depressed significantly for the first minute after a 2-s MVC ($P = 0.005$). In this period, F-wave area was reduced by $26.6 \pm 17.6\%$ of control value. After a 2-min MVC, depression of F-wave area over the first minute was more pronounced (by $48.2 \pm 19.9\%$), and the responses remained depressed for a further ~ 9 min ($P < 0.045$; Fig. 4B). Comparison of corresponding time points between the contractions revealed differences at 2–8 min (Student–Newman–Keuls; $P < 0.016$; Fig. 4B). As in study 2, the persistence of the F-wave was high and reproducible prior to contractions (Fig. 4C). Two-way ANOVA showed a significant effect of contraction duration ($F_{1,7} = 12.502$, $P = 0.01$), and time ($F_{6,42} = 16.01$, $P < 0.001$), and an interaction ($F_{6,42} = 2.785$, $P = 0.023$). F-wave persistence declined by $17.7 \pm 13.3\%$ of control after a 2-s MVC and on *post hoc* testing remained significantly decreased for 1 min ($P < 0.001$; Fig. 4C). After a 2-min MVC, persistence decreased by $34.6 \pm 18.0\%$ and remained significantly reduced for 8 min ($P < 0.046$; Fig. 4C). Comparison of corresponding time points of the two contractions showed significant differences from immediately after the MVCs to ~ 4 min and at 7 min post-contraction (Student–Newman–Keuls; $P < 0.03$).

Study 4: M-waves at rest after a 2-s and a 2-min MVC in ADM

This study investigated whether changes in motor axonal excitability during a sustained MVC contribute to the observed depression in F-wave area. When examined at rest after a 2-s MVC, submaximal M-waves evoked

grouped into sets of 30. There was an overall effect of time on F-wave area and the hash (#) indicates sets that are significantly lower than the combined baseline values ($P < 0.05$). C, group data showing F-wave persistence in the relaxed muscle before and after a 10-s (open circle) and a 1-min (filled circle) MVC. F-wave persistence decreased (by $14.6 \pm 10.5\%$ and $32.5 \pm 17.1\%$ after 10-s and 1-min MVCs, respectively). The asterisks (*) indicate data points that are significantly lower than the baseline values for each contraction ($P < 0.05$). The daggers (†) indicate differences between contractions at specific times ($P < 0.002$).

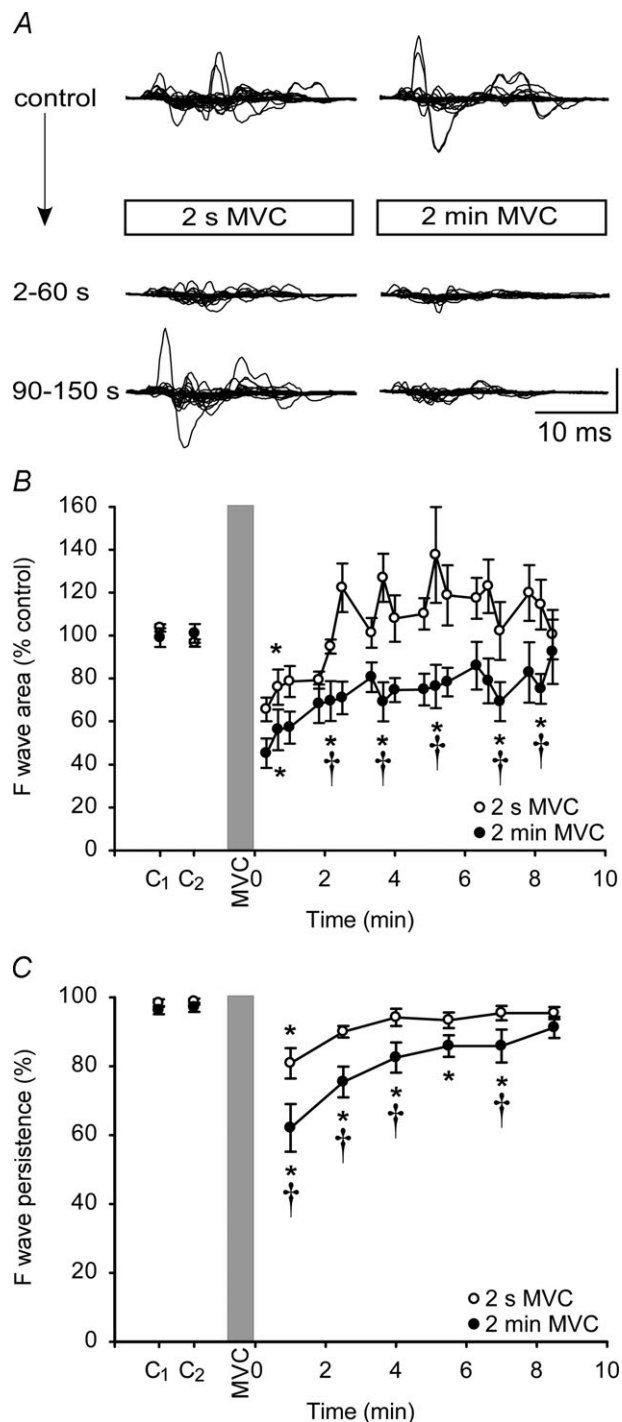


Figure 4. F-waves in ADM after MVCs of 2 s and 2 min duration

A, F-waves recorded from ADM at rest before and after a 2-s (left) and a 2-min (right) sustained MVC for a single subject. In each panel, top traces represent F-waves recorded before the MVC and are shown superimposed. Lower traces show superimposed responses for the first and second sets after the MVC. Each set consisted of 30 F-waves. Vertical calibration: 0.2 mV. B, group data ($n = 8$; means \pm SEM) showing changes in the area of F-waves after a 2-s (open circles) and a 2-min sustained MVC (filled circles). For illustration, each circle represents the average area of 10 consecutive

by ulnar nerve stimulation behaved differently from F-waves. The area of the M-wave evoked by the previously defined *maximal* stimulus intensity increased by $4.9 \pm 2.74\%$ of control after a 2-s MVC ($F_{1.5,10.7} = 6.8$, $P = 0.02$) and remained elevated for another ~ 6 min ($P < 0.01$; Fig. 5A). However, M-waves did not show significant changes after a 2-s MVC when examined at two *submaximal* stimulus intensities to produce 50% Mmax ($F_{1.6,11.2} = 1.55$, $P = 0.25$) and 25% Mmax ($F_{1.9,13.2} = 1.51$, $P = 0.26$) (Fig. 5A). After a 2-min MVC, maximal M-wave area increased by $22.81 \pm 19.97\%$ of control ($F_{1.5,10.2} = 0.03$; Fig. 5B) and on *post hoc* testing remained significantly elevated for 1 min ($P = 0.013$). In contrast, when tested at submaximal intensities, M-waves were significantly depressed by $\sim 50\%$ of control for the first minute after a 2-min MVC ($F_{2.2,15.6} = 3.96$, $P = 0.03$ and $F_{1.5,10.4} = 2.42$, $P = 0.043$ for 50 and 25% Mmax, respectively).

Discussion

This study provides the first evidence that volitional activity produces a graded reduction in the susceptibility of human motoneurons to discharge a recurrent action potential. When measured at rest immediately after a sustained MVC, the mean area and probability of occurrence of F-waves (*F persistence*) are decreased in both ADM and TA. When F-waves in ADM were examined after a very short, 2-s MVC and a very long, 2-min MVC, the magnitude and time course of the depression of mean area and persistence were significantly greater after the longer contraction. Although the depression of area was similar after a 10-s or a 1-min MVC, for ADM the reduction in persistence of the F-wave was greater after the longer contraction. These differences suggest that, at least for ADM, the depression of the F-wave, which is seen as a decrease in persistence and mean area, is related to the duration of the voluntary activity and that it may represent a decrease in motoneuronal excitability which could contribute to fatigue. In contrast, for TA, a relationship between the depression of the F-wave after an MVC and the duration of activity is not clear. Submaximal M-waves evoked in ADM were also depressed by a 2-min MVC but not by a 2-s MVC. The different time

F-waves for each subject. For statistical tests, F-waves were grouped into sets of 30. C, group data showing F-wave persistence in the relaxed muscle before and after a 2-s (open circle) and a 2-min (filled circle) MVC. There was an effect of contraction duration on both area and persistence of the F-waves. Asterisks (*) indicate that values for the set of 30 F-waves are significantly lower than the baseline value for the contraction ($P < 0.05$). Daggers (†) indicate differences between contractions at specific times ($P < 0.05$). The shaded vertical bar denotes the MVC.

course for development of F-wave and M-wave depression combined with the supramaximal stimuli used to evoke F-waves suggest that sites other than the site of stimulation are responsible for the observed depression of F-waves after an MVC.

F-waves were first described in humans by Magladery & McDougal (1950). When a supramaximal electrical stimulus is applied to a motor nerve, impulses propagate in both directions, orthodromically towards the muscle and antidromically towards the cell bodies of the motoneurons. Antidromic excitation causes backfiring of a proportion of motoneurons (e.g. Renshaw, 1941;

Lloyd, 1943), and the subsequent orthodromic potentials which can be recorded from a target muscle are known as F-waves (e.g. Dawson & Merton, 1956; Thorne, 1965; Mayer & Feldman, 1967). There are a number of potential sites at which alterations in function might decrease the size and/or persistence of F-waves after an MVC. First, F-waves will decrease if the same electrical stimulus activates a smaller number of axons at the site of stimulation after activity. Second, changes at the axon initial segment (or first node of Ranvier) might contribute to the decrease in F-waves either by blocking the antidromic potential due to local hyperpolarisation or by blocking the recurrent orthodromic potential (i.e. recurrent wave) and re-depolarisation of the axon. Finally, altered motoneuronal excitability at the soma might contribute to the observed decrease in F-waves after activity. In each of these cases, decreases in area and persistence of the F-wave would both reflect a reduction in the average number of motoneurons that generated recurrent potentials in response to each stimulus. In the following sections, we will discuss these possibilities.

A reduction in the number of axons activated by stimulation after activity is unlikely in the current study. We used supramaximal stimulation (150% of the intensity to elicit Mmax) to evoke F-waves to overcome any activity-dependent changes in axonal excitability at the site of stimulation, and our results show that maximal M-waves were not depressed after the sustained maximal contraction (Fig. 5A and B). However, there are known changes in axonal threshold with voluntary activity (e.g. Vagg *et al.* 1998) and such changes were confirmed here by the depression of submaximal M-waves (Fig. 5A and B). Although the changes in the current study were not as long lasting as those described for the median-innervated thenar muscles (Vagg *et al.* 1998), various differences in excitability parameters between motor axons in the ulnar and median nerves have been reported (Bae *et al.* 2009; Murray & Jankelowitz, 2011). The changes in axonal threshold reflect activity-dependent hyperpolarisation of the peripheral motor axons as a consequence of activation of the electrogenic Na⁺-K⁺ pump (e.g. Schoepfle & Katholi, 1973; Bostock & Grafe, 1985). The hyperpolarisation usually has little effect on normal action potential propagation, with only a small slowing of conduction velocity, except at regions where the safety margin for conduction is low (e.g. Kuwabara *et al.* 2002; Noto *et al.* 2011). However, in demyelinated axons, activity can cause conduction block (Bostock & Grafe, 1985; Cappelen-Smith *et al.* 2000). For antidromic action potentials, the junction of the myelinated axon with the unmyelinated axon initial segment is a region of low safety margin (Coombs *et al.* 1957a) so that axonal activity-dependent hyperpolarisation might depress F-waves by block at this site. However, in the present study, the changes in submaximal M-waves did

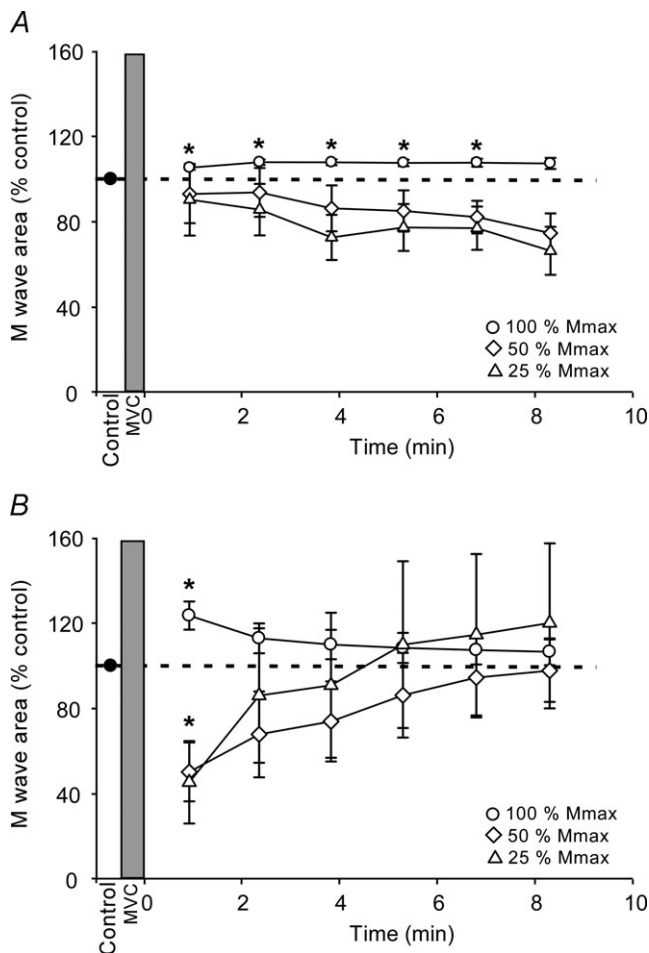


Figure 5. Maximal and submaximal M-waves in ADM after MVCs of 2 s and 2 min duration

Pooled data ($n = 8$; means \pm SEM) showing changes in the area of M-waves in ADM at rest after a 2-s (A) and a 2-min (B) sustained MVC. Electrical stimuli were delivered in sequence to the ulnar nerve at regular intervals to evoke M-waves of 100% (circles), 50% (diamonds) and 25% (triangles) of Mmax in ADM. Each data point represents the average area of 10 M-waves. M-wave area was normalised to the mean value of the control sets. The dotted line represents the mean value of the control sets before MVC. The shaded vertical bar denotes the MVC. Asterisks (*) indicate significant changes from the baseline values for each contraction ($P < 0.05$).

not match the time course of the depression of F-wave area as there was no apparent depression of axonal excitability with a 2-s MVC. This mismatch suggests that the F-wave depression is not simply the result of the same activity-dependent changes which occur in the peripheral motor axons, although a similar mechanism affecting the axon initial segment with a different time course cannot be ruled out.

Action potentials are initiated and modulated at the axon initial segment and their rapid propagation occurs along the axon via the nodes of Ranvier in myelinated axons (e.g. Araki & Otani, 1955; Coombs *et al.* 1957*b*; Kole & Stuart, 2008; Clark *et al.* 2009; Kress & Mennerick, 2009). The axon initial segment and the nodes of Ranvier of the motor axon are distinct specialised axonal domains and their complement and distribution of channels differ. The axon initial segment is enriched with voltage-gated sodium (Nav1.1, Nav1.2, Nav1.6), potassium (Kv1.1, Kv1.2) and calcium (Cav2.1, Cav2.2, Cav3) channels (Westenbroek *et al.* 1989; Van Wart *et al.* 2007; Duflocq *et al.* 2008, 2011; Lorincz & Nusser, 2008; Ogawa & Rasband, 2008; Bender & Trussell, 2009; Hu *et al.* 2009; Debanne *et al.* 2011), whereas Nav1.6 is the major sodium channel at mature nodes of Ranvier (Caldwell *et al.* 2000; Boiko *et al.* 2001). Moreover, there is a differential distribution of sodium and potassium channels within the initial segment. Nav1.6, Kv1.1 and Kv1.2 are predominantly localised distally at the likely site of action potential generation, with Nav1.2 and Nav1.1 in the proximal part of the initial segment (Boiko *et al.* 2003; Inda *et al.* 2006; Van Wart *et al.* 2007; Hu *et al.* 2009; Duflocq *et al.* 2011). Nav1.6 channels have a low threshold and contribute to action potential initiation, while the proximal Nav1.2 channels have a higher threshold and promote back-propagation (Hu *et al.* 2009). Furthermore, calcium influx produced by activity at the axon initial segment is also likely to affect spike initiation (Bender & Trussell, 2009). Because of the different distribution of ion channels in the initial segment and the nodes, it is likely that these regions respond differently to repetitive activity. However, no studies have yet investigated the excitability of the axon initial segment during repetitive firing in motoneurons. Therefore, it is not possible to speculate further on whether the depression of F-waves after as little as 2 s of voluntary activity represents altered function of this important region.

In humans, findings such as the depression of cervicomedullary motor evoked potentials (CMEPs) during a sustained MVC suggest a decrease in motoneurone excitability with contractions of ~10 s (McNeil *et al.* 2009, 2011). However, there is no evidence in humans for depression of motoneurone excitability after only 2 s of contraction. In animals, repetitive activation has been shown to reduce the responsiveness of motoneurons. During sustained activation of motoneurons by intracellular or

extracellular stimulation, many cells which initially fired repetitively stop firing or reduce their firing rate (Kernell & Monster, 1982; Spielmann *et al.* 1993; Sawczuk *et al.* 1997; see also Peters & Fuglevand, 1999). This spike frequency adaptation has early and late phases. It can continue throughout the duration of firing which may be several minutes and is more prominent in the larger more fatigable motor units (Kernell & Monster, 1982; Spielmann *et al.* 1993). Motoneurons recover within 1–2 min after the cessation of stimulation. The first few hundred milliseconds of this process reflects slow inactivation of sodium channels, while the mechanism of late adaptation remains uncertain (Miles *et al.* 2005; Brownstone, 2006). Thus, depression of F-waves after a sustained maximal contraction may be due to decreased excitability of the motoneurons, which makes the soma harder to excite through antidromic invasion.

Although F-wave area and persistence were reduced after 10-s and 60-s MVCs in both the intrinsic hand muscle (ADM) and in the ankle dorsiflexor muscle (TA), the depression was somewhat different in the two muscles. In TA, depression of both area and persistence of F-wave were identical after the two contractions, whereas in ADM, persistence differed significantly although area did not. Thus, while it seems likely that the depression of the F-wave in TA and ADM after MVCs occur through similar activity-dependent mechanisms, the change in the F-wave in TA was not altered by a sixfold increase in duration of activity whereas that in ADM was. We can only speculate that this difference represents a difference in the properties of the motoneurons in the two pools. Differences in the behaviour of responses to corticospinal tract stimulation after MVCs has been reported for TA compared to another intrinsic hand muscle (first dorsal interosseous; Giesbrecht *et al.* 2011) as have differences in activity-dependent hyperpolarisation of the motor axons in the peroneal (lower limb) and median (upper limb) nerves (Kuwabara *et al.* 2002).

Although the depression of F-waves provides evidence of changes intrinsic to the motoneurons following repetitive voluntary activity, the functional significance of the finding is not clear. It may depend on the precise site of impairment. If the F-wave depression reflects decreased excitability primarily at the soma, then the motoneurons would also have reduced responses to synaptic input, i.e. their gain is reduced. This is consistent with the view that human motoneurons require progressively increasing synaptic drive to maintain a constant firing rate in a sustained voluntary contraction (Johnson *et al.* 2004). If the axon initial segment is altered, then the mechanism could affect action potential initiation, which could also alter the input–output function of the motoneurons. Alternatively change at the axon initial segment may only affect antidromic action potentials with no impairment

of orthodromic potentials. Such a change could still affect physiologically back-propagating action potentials, which could influence overall excitability as well as synaptic efficacy of the motoneurons. As the first node of Ranvier is important for high-frequency bursts in some neurones (Kole, 2011) a decrease in generation or transmission of recurrent potentials may affect the generation of doublets. However, the role of the first node is not clear in motoneurons. There are instances in which the firing rate of the motoneurone soma does not match that of the axon (see Gogan *et al.* 1984)

In summary, we found a strong depression of F-waves at rest after brief and sustained maximal voluntary contractions. The magnitude and the time course of the depression depended on the duration of the activity for ADM muscle, which suggests that the reduction of F-waves could be associated with the central fatigue accompanying voluntary exercise. The changes in F-waves cannot simply be explained by altered excitability of the peripheral motor axons. Thus, we propose that activity-dependent changes intrinsic to the motoneurons, occurring at the axon initial segment or soma, are responsible for the depression. If these changes affect the gain of the motoneurone pool, then our results highlight the need for compensation by descending and reflex inputs in order to maintain motoneuronal output.

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Note added in proof

Similar changes in F waves with voluntary activity have been reported by Rossi et al. Rossi A, Rossi S & Ginanneschi F. (2012). Activity dependent changes in intrinsic excitability of human spinal motoneurons produced by natural activity. *J Neurophysiol* (in press).

Author contributions

All authors contributed to all aspect of the study and all approved the final version of the manuscript. All experiments were performed at Neuroscience Research Australia in Sydney, Australia.

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