Discharge Properties of Motor Units of the Abductor Hallucis Muscle During Cramp Contractions

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In this study, we analyzed the behavior of motor units during cramps elicited by electrical stimulation of the muscle motor point (Minetto et al. 2008; Minetto and Botter 2009). The aim of the study was to assess the degree of variability and the degree of common oscillations in motor unit discharges during cramps.

INTRODUCTION

A muscle cramp is a sudden, involuntary, and painful contraction of a muscle or part of it, self-extinguishing within seconds to minutes, often accompanied with a palpable knotting of the muscle (AAEM Glossary of Terms 2001; Miller and Layzer 2005). The experimental study of cramps is complicated by their unpredictable occurrence. However, physiological (Jung et al. 2005; Roeleveld et al. 2000; Ross and Thomas 1995), electrical (Bertolasi et al. 1993; Minetto et al. 2008; Stone et al. 2000), and magnetic (Caress et al. 2000) procedures have been proposed to standardize cramp induction under laboratory conditions.

The origin of cramps has been long discussed (Layzer 1994; Miller and Layzer 2005). Cramps may result from abnormal excitation of the terminal branches of motor axons (peripheral origin) (Bertolasi et al. 1993; Roeleveld et al. 2000) or from hyperexcitability of motor neurons (central origin) (Ge et al. 2008; Khan and Burne 2007; Norris et al. 1957; Obi et al. 1993; Ross and Thomas 1995; Serrao et al. 2007). Although both hypotheses fail to explain all the observations, the analysis of motor unit behavior during cramps indicates that the synaptic inputs the motor neurons receive likely play a role in cramp development and extinction. For example, the decrease in discharge rate over time during cramp may be associated with reflex inhibition of the motor neuron pool (Ross and Thomas 1995) because it also occurs during sustained voluntary contractions (Bigland-Ritchie et al. 1986; Nordstrom et al. 2007). Moreover, muscle activity generated during cramp and voluntary contractions can be equally inhibited by electrical stimulation of tendon afferents (Khan and Burne 2007).

In accord with these observations, a possible model of cramp development that involves spinal pathways consists of a positive feedback loop, in which the motor neurons receiveafferent inputs, resulting in increased intrinsic excitability (Ross and Thomas 1995).

Although the electrodiagnostic definition of a cramp is an involuntary repetitive discharge of motor unit action potentials at high rate and in a large area of the muscle (AAEM Glossary of Terms 2001), the high values of discharge rate and discharge variability during cramps are usually indirectly inferred from the surface electromyographic (EMG) amplitude (Roeleveld et al. 2000). A direct measure of discharge characteristics of a pool of motor units during cramp contractions has been reported in only few studies (Norris et al. 1957; Ross and Thomas 1995). Moreover, there are no studies that investigated the relationships between discharges of different motor units during cramp development. If spinal pathways are involved in cramp development, it may be hypothesized that during cramps the oscillations in discharge of individual motor units are partly correlated because the afferent input in the positive feedback loop is probably similar to a large number of motor neurons.

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METHODS

Subjects

Eleven subjects (two women and nine men; age: mean ± SD, 27.0 ± 3.7 yr) volunteered to participate in the study. Subjects were free from neuromuscular or skeletal impairments. They were asked to refrain from performing strenuous physical activity for 24 h before each experimental session. The subjects received a detailed explanation of the study and gave written informed consent prior to participation. The study conformed with the guidelines in the Declaration of Helsinki and was approved by the local ethics committee (N-20080010).

EMG recordings

Bipolar surface EMG signals were detected from the abductor hallucis muscle with a 6 × 5 grid of electrodes (2-mm diameter, 5-mm interelectrode distance; LISiN, Turin, Italy). The grid was placed between the muscle motor point and the distal tendon and was aligned with the longitudinal axis of the muscle (Fig. 1). Before placement of the grid, the skin was lightly abraded with abrasive paste. To ensure proper electrode–skin contact, 10 μl of conductive gel was inserted into the electrode cavities of the grid with a gel dispenser. The bipolar surface EMG signals were amplified (multichannel surface EMG amplifier, EMG-USB, LISiN-OT Bioeletronica, Turin, Italy), band-pass filtered (3-dB bandwidth, 10–750 Hz), sampled at 2,048 samples/s per channel, A/D converted on 12 bits, displayed on-line, and stored for further analysis.

Intramuscular EMG signals were detected with two pairs of wire electrodes made of Teflon-coated stainless steel (A-M Systems, Carlsborg, WA) that were inserted into the muscle with a 25-G needle between the motor point and the top edge of the surface grid (Fig. 1). The needle was inserted to a depth of a few millimeters below the muscle fascia and removed to leave the wire electrodes inside the muscle. The insulated wires were cut to expose only the cross section and provided one bipolar signal, which was amplified (Dantec Counterpoint electromyograph, Dantec Medical A/S, Skovlunde, Denmark), band-pass filtered (500 Hz to 5 kHz), sampled at 10 kHz, and stored after 12-bit A/D conversion. A reference electrode for both the surface and intramuscular recordings was placed around the ankle.

Electrically elicited muscle cramps

Cramps were elicited in the abductor hallucis muscle of the dominant foot with electrical stimulation of the muscle motor point, as described previously (Minetto et al. 2008; Minetto and Botter 2009). The subject was seated comfortably on a chair with the ankle in neutral position and the foot resting on a padded support. The muscle motor point was identified with a pen electrode (small size cathode: 1-cm² surface; Globus Italia, Codognè, Italy) and corresponded to the location generating the maximal mechanical response with the minimum injected current. Electrical stimulation was provided by a programmable multichannel neuromuscular stimulator (LISiN) with a hybrid output stage. An adhesive stimulation electrode (10 × 10 mm; Spes Medica, Battipaglia, Italy) was placed over the identified motor point and a larger electrode (50 × 80 mm) was placed over the lateral side of the foot to close the stimulation current loop (monopolar stimulation).

Bursts of 150 rectangular stimuli (152-μs duration each) at current intensity 30% higher than that eliciting the maximal M-wave were delivered at frequencies increasing from 4 Hz at increments of 2 Hz until the cramp was elicited. A resting period of 1 min separated each burst of stimulation. The presence of a cramp was assessed by subject feedback, clinical observation of a continuous muscle contraction that persisted after the cessation of the stimulation, and the presence of involuntary EMG activity after cessation of the stimulation. The frequency of the burst that elicited a muscle cramp was defined as the cramp threshold frequency.

Procedures

Each subject participated in two experimental sessions on 2 days. During the first day, surface EMG signals were acquired during the procedure for determining the cramp threshold frequency. During the second day, a cramp was elicited by applying a burst of 150 stimuli at a frequency corresponding to twice the cramp threshold frequency (Minetto et al. 2009), as determined in the first day. Five minutes after the elicitation of the cramp, the same stimulation procedure was applied to elicit a second cramp to investigate the change in susceptibility to cramps with repetitive cramp generation. Locations of the stimulation and recording electrodes were marked on the first day and reproduced in the second day. In the second experimental day, surface and intramuscular EMG signals were concurrently recorded during the first cramp, whereas only surface EMG signals were recorded during the second cramp.

The subject was asked to keep the muscle relaxed both before and after the stimulation period, to report immediately when he/she felt a muscle cramp and to let the cramp develop until it disappeared spontaneously without any resistance. A 0–10 visual analogue scale

FIG. 1. Electrode placement on the abductor hallucis muscle. The electrode grid used for surface electromyographic (EMG) detection was located between the selected motor point, where the stimulation electrode was placed, and the distal tendon. Intramuscular EMG signals were detected with two pairs of wire electrodes, inserted between the stimulation electrode and the surface grid. The positions of the large electrode used to close the stimulation current loop, of the reference electrode, and of the skin thermistor are also shown.
(VAS; 0 represented “no pain” and 10 represented “intolerable intense pain”) was used to quantify the pain associated with development of the cramp. Skin temperature was monitored throughout the experiments with a skin thermistor (Omega Engineering, Stamford, CT) located next to the stimulation electrode.

**Signal analysis**

Twenty-five (5 x 5) bipolar surface EMG signals were recorded with the electrode grid along the direction of the muscle fibers. The interval of time during which the muscle was active during cramp development was identified from the surface EMG using an algorithm for the detection of muscle onset of activity (Merlo et al. 2003). The EMG average rectified value (ARV) was computed from each bipolar recording for intervals of 1-s duration. The 23 values of ARV were averaged across the grid to obtain an estimate of the intensity of muscle activity, whose maximum value across the time intervals was used for statistical analysis.

Action potentials of individual motor units were identified and classified from the intramuscular EMG signals with a decomposition algorithm (McGill et al. 2005). The algorithm includes a user interface for manually editing and verifying the results. The software displays a segment of the intramuscular EMG signal, the templates of the action potentials of the identified motor units, the discharge patterns, and a close-up of the signal for resolving missed discharges and superimpositions. The automatic decomposition was manually verified by two expert operators.

From the identified motor unit spike trains, the SD of the interspike interval (ISI), which is an index of the absolute variability in discharge rate, and the coefficient of variation (CoV, SD divided by mean value in %) for the ISI were computed over time from consecutive sets of 30 discharges.

Moreover, to study the modulation in discharge, the instantaneous discharge rates were smoothed by low-pass filtering (fourth-order Butterworth filter; cutoff frequency, 2 Hz) (De Luca et al. 1982). To identify common oscillatory components, the smoothed discharge rates of motor unit pairs were analyzed with coherence spectra after removal of the mean value (Myers et al. 2004). The coherence spectra were computed from intervals of 2-s duration during the period of activation of each motor unit pair. Peaks in the coherence functions were considered significant when they exceeded the 95% confidence interval. Common oscillations in the smoothed discharge rates were also investigated in the time domain with cross-correlation (CC) analysis (De Luca et al. 1982, 2009). The CC function between pairs of motor unit discharge rates was computed from intervals of 4-s duration, after removal of the mean, for the entire period during which the motor units in the pair were concurrently active. The peak value and the time lag corresponding to the peak of the CC function indicated the strength and delay of the association between the discharge rate oscillations. For the motor unit pairs that showed significant CC for the entire period of activation, a cross time–frequency transformation (Choi–Williams distribution, parameter sigma = 0.5; Cohen 1995) was also applied to investigate the time course of the common oscillations in discharge rate.

**Statistical analysis**

The Wilcoxon signed-rank sum test was used to compare the peak and minimum values of the discharge rate and the CoV for the ISI in the first and last 2 s of activity. The same test was also used for comparing cramp durations, surface EMG amplitudes, and skin temperature values between the two cramps of the second day. Friedman’s ANOVA followed by Dunn’s post hoc test were used for analyzing the time course of both the discharge rate values and the number of doublets (relative to the total number of discharges) across the motor unit activation interval. The Spearman rank correlation analysis was used to test for linear correlation between successive ISIs [nth ISI vs. (n + 1)th ISI] (Berg et al. 2007) and between the SD and the mean of the ISI for each motor unit. Data are expressed as mean ± SD or median and range, as indicated. Threshold for statistical significance was set to $P = 0.05$.

**RESULTS**

In the first day, it was possible to evoke a cramp in the abductor hallucis muscle of all subjects. These cramps were subjectively reported as moderately painful (VAS ratings of pain ranged from 2 to 4) and evoked sensations identical to those experienced during spontaneous cramps. The median (range) value of the cramp threshold frequency was 12 (8–20) Hz.

In the second day, it was possible to evoke the first cramp in all subjects, whereas the electrical stimulation method did not evoke the second cramp in two subjects. The skin temperature did not change between the first and the second trials (29.2 ± 1.0 vs. 29.1 ± 1.6°C; $P = 0.21$). The first cramp elicited in the second day started 4.5 ± 1.5 s before the end of the stimulation burst in 10 of the 11 subjects and 15 s after the end of the stimulation in one subject, as estimated from the surface EMG.

**TABLE 1. Stimulation frequency, stimulation current intensity, cramp duration, and peak average rectified value (ARV) of the surface EMG for the two cramps elicited in the second day**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Stimulation Frequency, Hz</th>
<th>Current Intensity, mA</th>
<th>Day 1, First Cramp</th>
<th>Day 2, Second Cramp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Duration, s</td>
<td>Peak-ARV, µV</td>
</tr>
<tr>
<td>1</td>
<td>24</td>
<td>30</td>
<td>28.0</td>
<td>47.3</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>24</td>
<td>47.0</td>
<td>82.6</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>32</td>
<td>26.0</td>
<td>87.1</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>24</td>
<td>22.0</td>
<td>62.7</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>20</td>
<td>33.0</td>
<td>82.2</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>39</td>
<td>81.0</td>
<td>103.0</td>
</tr>
<tr>
<td>7</td>
<td>40</td>
<td>39</td>
<td>51.0</td>
<td>53.0</td>
</tr>
<tr>
<td>8</td>
<td>28</td>
<td>25</td>
<td>15.0</td>
<td>43.9</td>
</tr>
<tr>
<td>9</td>
<td>24</td>
<td>28</td>
<td>18.0</td>
<td>66.2</td>
</tr>
<tr>
<td>10</td>
<td>24</td>
<td>20</td>
<td>10.0</td>
<td>65.6</td>
</tr>
<tr>
<td>11</td>
<td>20</td>
<td>26</td>
<td>14.0</td>
<td>49.0</td>
</tr>
<tr>
<td>Mean (±SD)</td>
<td></td>
<td></td>
<td>31.4 (±21.0)</td>
<td>67.5 (±19.1)</td>
</tr>
</tbody>
</table>

In the first session, the cramp started during the stimulation burst in 10 of 11 subjects, whereas in the second session all cramps started during the course of the stimulation burst. The cramp duration was estimated from the end of the stimulation in all cases. The symbol “—” indicates that the second cramp could not be elicited. *Significant difference between durations of the two cramps (Wilcoxon test, $P < 0.01$).
signals; when it could be elicited, the second cramp always started during the stimulation burst (3.0 ± 1.0 s before the end of the stimulation). Because the time interval between the initiation of the cramp and the end of the stimulation was small and it was not possible to identify single motor unit activities before the end of the stimulation (due to the presence of stimulation artifacts between consecutive stimuli that were very close to each other), in the following results the characteristics of the cramps are referred to the end of the stimulation, which was considered as approximately corresponding to the beginning of the cramp.

Table 1 shows the stimulation parameters, cramp duration (starting from the end of the stimulation burst), and surface EMG amplitude for the two cramps elicited in the second day. Figure 2 shows an example of surface EMG signals detected during the two cramps in one subject. In this example, the duration of the first cramp (~30 s from the end of the stimulation) was longer than the duration of the second cramp.

**FIG. 2.** Temporal development of surface EMG during 2 cramps elicited 5 min apart in one representative subject, starting from the end of the stimulation burst (time 0 indicates the end of stimulation). The 5 bipolar EMG channels of the central column of the surface electrode grid are reported for both cramps.
FIG. 3. Intramuscular EMG recordings during the first (A) and last (B) 2 s of activation of a motor unit. The action potentials (APs) of the motor unit are also shown when detected in the first and last 2 s of activation. The similarity in shape and amplitude of the APs indicates that they were probably generated by a single unit. C and D: the instantaneous and smoothed discharge rate for the representative motor unit for the entire duration of its activation interval (time 0 indicates the end of stimulation). E–H: same as in A–D for another representative subject, in which the cramp onset occurred about 15 s after the end of the stimulation burst.
(−5 s from the end of the stimulation). From the group data, the duration of the first cramp was longer than that of the second cramp (31.4 ± 21.0 s vs. 11.7 ± 16.3 s; P < 0.01), whereas the peak EMG amplitude was not significantly different between the two cramps (67.5 ± 19.1 vs. 53.1 ± 22.2 μV; P = 0.37).

Motor unit discharge rate

The motor unit behavior was analyzed for only the first cramp of the second day, during which intramuscular EMG signals were recorded. Figure 3 shows the behavior of two motor units activated during the first cramp of the second day in two subjects. These motor units were active for about 40 s during which their action potentials could be accurately tracked. Their discharge rate initially increased for about 5–10 s and then decreased to a value of nearly 6 pulses/s (pps) before the units were deraught. A similar behavior was observed for all the identified motor units, as seen from the following group data analysis.

In all, 48 motor units were identified (4.4 ± 1.7 motor units per subject, range 2–7) from the intramuscular EMG recordings. The average interval of activation of the identified motor units was 23.6 ± 16.2 s. The average discharge rate over the interval of activity (n = 48 motor units) was 14.5 ± 5.1 pps. However, the peak rate had a large variability across units (25.0 ± 8.0 pps; range 13.9–46.7 pps). A relatively small proportion of motor units (6/48) had peak rates >30 pps. All motor units (n = 48) reached the peak in discharge rate within the first 30% portion of the activation time (mean value for the 48 motor units: 14 ± 11%). As shown representatively in Fig. 3, after reaching the peak, the motor unit discharge rate decreased during the recordings and reached the minimum in the last 2 s of activation (6.0 ± 0.8 pps; range 4.9–8.4 pps; significantly different from the peak, P < 0.001). The relative decrease in discharge rate from the peak to the minimum value was 74.0 ± 7.9% (range in decrease, 57.3–87.0%), with an average rate of change (calculated as relative decrease in discharge rate divided by the time interval between peak and minimum) of −7.8 ± 7.4%/s (range −1.6 to −28.6%/s).

Figure 4 shows the mean discharge rates at each 10% portion of the activation interval for the identified motor units (n = 48). The discharge rates in the first four intervals were greater than those in the last four intervals of activity (P < 0.01). Moreover, the fifth interval corresponded to greater rates than those of the last three (P < 0.01).

Discharge rate variability

As can be observed in Figs. 3 and 5, the motor unit discharge rate had large variability. The average CoV for the ISI (n = 48 motor units) was 44.6 ± 9.7%, which is larger than that usually observed during voluntary contractions (Enoka et al. 1989; Macefield et al. 2000; Moritz et al. 2005). CoV for the ISI did not change (P = 0.14) during the interval of activity and it was 44.8 ± 13.5% (range, 6.6–80.4%) in the first 2 s and 40.7 ± 13.6% (range, 14.8–70.2%) in the last 2 s of activity. The high coefficient of variation corresponded to a low correlation between the nth and the (n + 1)th ISI value (R = 0.17 ± 0.18). In 38 motor units, the SD of ISI was positively correlated to the mean ISI (R² = 0.37 ± 0.19; range, 0.07–0.83, P < 0.05), with the slope of the linear regression 0.33 ± 0.12, whereas there was no significant association between mean and SD of the ISI in the remaining 10 motor units.

The large variability in discharge rate caused the occasional observation of discharges occurring at very short time interval from each other. Figure 5 shows a sample recording with examples of these discharges. Discharges occurring at time intervals <20 ms, often referred to as doublets (AAEM Glossary of Terms 2001; Bawa and Calancie 1983), represented 4.1 ± 4.7% (range, 0.2–19.1%) of the total number of discharges and were observed in 37 motor units. On average, 13.1 ± 26.0 (range, 1.0–155.0) doublets were identified per motor unit. There were 1.5 ± 1.8% (range, 0.1–7.1%) discharges with ISI <10 ms (instantaneous rate >100 pps) and these discharges were identified in 20 motor units. On average, 4.0 ± 5.9 (range, 1.0–27.0) discharges with ISI <10 ms were identified per motor unit. The relative proportion of doublets relative to the total number of discharges was maximum in the first 10% of time of activity and decreased over time (Fig. 5D; Friedman’s ANOVA, P < 0.0001). However, the number of discharges with ISI <10 or <20 ms was similar to the theoretical estimate obtained by assuming a Gaussian distribution of ISI with the observed mean and variability in ISI. For example, the expected number of ISIs <10 or <20 ms for a mean ISI of 50 ms (20 pps) and a CoV for ISI of 37% (within the range of the observed values) is roughly 1 and 5%, respectively, similar to the measured values. Thus discharges at very high instantaneous rate were determined by the high variability in discharge.

Modulation of discharge rate

Figure 6A shows the smoothed discharge rates for two motor units and Fig. 6B shows the coherence spectrum for this motor unit pair. In this example, the two discharge rates have significant coherence with two peaks at frequencies 0.8 and 1.7 Hz. When all the pairs of motor unit discharges were analyzed (96 motor unit pairs), the coherence spectra between smoothed discharge rates of 29 pairs showed one significant peak. For these motor units, the peak was located at 1.4 ± 0.4 Hz (range, 0.7–2.2 Hz) and had amplitude of 0.51 ± 0.24 (range, 0.17–0.95). Moreover, in 8 of the 96 motor unit pairs, the coherence spectrum between smoothed discharge rates showed two significant peaks (as in a representative example in Fig. 6B) that were located at 1.3 ± 0.5 Hz (range, 0.6–1.9 Hz) and 1.5 ± 0.5 Hz (range, 0.9–2.2 Hz) and had amplitudes of 0.61 ± 0.07 (range, 0.48–0.70) and 0.54 ± 0.06 (range, 0.46–0.62), re-
FIG. 5.  A: intramuscular EMG recordings in the first (left) and last (right) 5 s of activation of a representative motor unit, whose APs are shown superimposed to each other. B: example of 4 doublets of the motor unit shown in A. C: instantaneous discharge rate of the motor unit for the entire duration of its activation interval and relation between successive interspike intervals (ISIs) [n-th ISI vs. (n + 1)-th ISI]. D: number of doublets (mean and SD) relative to the total number of discharges at each 10% time of the activation interval during cramp contractions (n = 48 motor units).
DISCUSSION

Motor units active during cramp contractions showed a range of discharge rates similar to that observed during voluntary contractions, but larger variability in the ISI. Moreover, during the cramp, motor units showed common oscillations in discharge, although with larger delays with respect to those observed during voluntary contractions.

Discharge rate

The maximal discharge rates at which the abductor hallucis motor units were found to discharge during cramp contractions were comparable to those reported for voluntary contractions of muscles of the foot (Macefield et al. 2000) and leg (Bellemare et al. 1983; van Cutsem et al. 1997). In the assessment of the maximal discharge rates, we should account for the possibility that the peak discharge was not identified because the cramp started before the end of the stimulation, whereas the motor units could be identified only after the stimulation. However, it is unlikely that much greater discharge rates could be identified before the stimulation end. First, the cramp initiated on average only 4.5 s before the stimulation end; thus the major part of each cramp developed later and was analyzed by single-unit identification. Second, in one subject the cramp was elicited 15 s after the end of the stimulation and the peak discharge rates observed for the five motor units identified from this subject (range, 15–24 pps) were not substantially greater than those for the other subjects.

The observed derecruitment rate was in the range 5–8 pps and corresponds to the minimal rate at which motor neurons discharge action potentials in voluntary contractions (van Cutsem et al. 1997). Furthermore, the shape of the action potentials repeated consistently over time. These results indicate that it is unlikely that the detected action potentials were generated at the intramuscular terminal branches of motor neuron axons.
and support the hypothesis of involvement of spinal pathways in cramp development and extinction (Ross and Thomas 1995).

The discharge rates of the detected motor units were observed to decrease during the contraction. As in voluntary contractions (Bigland-Ritchie et al. 1986; Nordstrom et al. 2007), during cramps the decrease in discharge may be due to motor neuron adaptation (Nordstrom et al. 2007) and/or changes in afferent input over time. Consistently, motor unit discharge rates in the absence of afferent feedback do not decrease with sustained activation (Gandevia et al. 1990; Macefield et al. 1993).

Cramp development may be due to a positive feedback loop in which the motor neurons have increased excitability. It was previously shown that bursts of peripheral electrical stimulation may generate involuntary muscle activity (Blouin et al. 2009; Lagerquist et al. 2009). Peripheral electrical stimulation may activate sensory afferents that trigger the generation of persistent inward currents (PICs), which change the excitability of motor neurons (Collins et al. 2002; Gorassini et al. 2002; Heckman et al. 2008; Nordstrom et al. 2007). PIC activity modifies the relation between synaptic input and motor neuron output (Heckman et al. 2008), so that afferent inputs converging on the motor neurons are amplified and may produce self-sustained discharges.

**Discharge rate variability**

Although motor units active during cramp showed a range of discharge rates similar to that observed during voluntary contractions, the discharge variability was greater than that reported for voluntary contractions of muscles of the foot (Macefield et al. 2000) and hand (Enoka et al. 1989; Moritz et al. 2005). The high variability in the ISI resulted in the occasional occurrence of doublets, whose number decreased during cramp development in parallel to a trend (although nonsignificant) for a reduction in discharge variability. Although the estimation of variability in discharge may have been partly biased by the oscillations in average discharge rate, a larger variability with respect to voluntary contractions is also evident from the presence of discharges with high instantaneous rates, whose percentage could be theoretically explained by the measured variability. This result is also in agreement with the observations of Ross and Thomas (1995) who reported that the discharge variability was greater during cramp with respect to

**FIG. 7.** A and B: smoothed discharge rates of 2 representative pairs of motor units. C and D: the high-pass filtered (5th-order high-pass Butterworth filter, cutoff frequency 0.5 Hz) smoothed discharge rates. The gray rectangles correspond to the intervals where the cross-correlation (CC) function exhibited the highest peak (peak CC). E and F: CC functions as a function of time for a range of time lags. The color bar indicates the CC value (CC amplitude).
voluntary contractions, although in their study the ISI variability was not quantitatively assessed.

The variability in discharge rate is due to synaptic noise to the motor neuron and its interaction with the time course of the afterhyperpolarization phase (Calvin and Stevens 1967; Matthews 1996; Stein et al. 2005). Synaptic noise of the membrane voltage trajectory results from the integration of inhibitory and excitatory afferent inputs (Berg et al. 2007; Destexhe and Contreras 2006; Stein et al. 2005). The balanced increase in these two types of inputs increases the discharge variability, if the inputs are uncorrelated, by increasing the fluctuations in the resting membrane potential (Berg et al. 2007; Rudolph and Destexhe 2004; Rudolph et al. 2007). The observation that cramp contractions are associated with high ISI variability indicates that the synaptic noise is likely greater during cramp with respect to voluntary contractions. During voluntary contractions, the descending drive from supraspinal centers accounts for about 70% of the discharge rate, whereas 30% is explained by afferent input (Macefield et al. 1993). Thus during voluntary efforts the main source of input is excitatory. It is expected that contractions generated by only afferent input, such as cramps, are associated with larger synaptic noise since the excitatory and inhibitory inputs are more balanced. According to this hypothesis, the discharge variability of motor neurons without afferent input is smaller than that in normally innervated motor neurons (Macefield et al. 1993). During cramp contractions, the synaptic input to the motor neurons is exclusively due to afferent activity, which can be inhibitory and excitatory and thus generates greater noise if inhibitory and excitatory components are uncorrelated (Berg et al. 2007; Rudolph and Destexhe 2004; Rudolph et al. 2007).

Common modulations of discharge rates

The coherence analysis showed the presence of common oscillatory components in the discharge rate of pairs of motor units with a frequency of about 1 Hz. Accordingly, the discharge rates of pairs of motor units showed significant correlation when analyzed in the time domain. These observations further confirm the spinal origin of the observed action potentials and indicate sources of similar input to the population of motor neurons. Consistently, Sowman et al. (2007) showed that periodontal mechanoreceptor anaestheticization resulted in decreased coherence between motor unit discharges at 8 Hz and reduction of jaw physiological tremor, possibly due to reduction or removal of a common input to masseteric motor neurons from peripheral afferents. Common oscillations in motor neuron discharge rate have also been documented during voluntary contractions in both the time and frequency domain (with peak in coherence in the frequency range 1–3 Hz) (De Luca and Erim 2002; De Luca et al. 1982, 2009; Erim et al. 1996). However, the common oscillations of discharge seen during voluntary contractions are in-phase and thus the relative time delay is close to zero (De Luca et al. 1982, 2009). On the contrary, the latency among the motor unit discharges observed in this study was in the range 0–230 ms. This observation does not agree with the presence of a common input to the motor neuron population during the cramp contractions because the delays observed are too large to be explained by different interneuronal pathways for different motor neurons. However, the results may be in agreement with the spatial development of the cramp in the muscle (Roeleveld et al. 2000), which may imply activation of muscle afferents in different time intervals. According to this view, it may be that different muscle portions are activated in different time instants (Roeleveld et al. 2000) that generate similar afferent activity, but delayed in time, which projects to different motor neurons.

There are several afferent pathways that may be involved in cramp contractions, including muscle spindles, muscle mechano- and metaboreceptors, and nonmuscle (cutaneous, tendon) afferents. Although it is not possible to identify relative contributions, the present data indicate that during cramp development the motor neurons receive input resulting from the combination of uncorrelated inhibitory and excitatory afferent sources summing with each other since the resulting input is associated with high noise. This input is similar for the motor neurons that exceed their activation threshold, but temporally delayed probably due to the spatial evolution of the cramp.

Cramp induction by repetitive electrical stimulation

Five minutes after the elicitation of the first cramp, the same stimulation procedure was applied to elicit a second cramp. The number of cramps elicited in the second trial and their duration were reduced compared with the first trial. This observation is in agreement with the anecdotal evidence that cramps do not occur soon after a first cramp episode and with the previously reported increase in the cramp threshold frequency between consecutive sessions of electrical stimulation (Minetto et al. 2008). Change in motor neuron excitability as a consequence of the first cramp may explain these observations. For example, PIC activity may decrease after the first cramp because the state of the channels mediating PICs is modified by their activation history (Nordstrom et al. 2007). Alternatively, hyperpolarization of sensory axons could have occurred following the first cramp due to activation of the electrogenic Na⁺-K⁺ pump to correct the intracellular Na⁺ accumulation that occurs when an axon conducts trains of impulses (Burke et al. 2001). Axonal hyperpolarization determines a short-term decrease in axonal excitability, thus reducing the afferent input.
converging on the motor neurons during and after cessation of the stimulation.

In conclusion, motor neurons of the abductor hallucis muscle discharge action potentials during cramp contractions at rates comparable with those reported for voluntary contractions, but with larger variability in the ISI. The high discharge variability is related to the afferent inputs that motor neurons receive, which can be inhibitory and excitatory, and thus generate greater noise than that in voluntary contraction. Moreover, the oscillations in discharge rate of the motor units active during cramp are correlated and coherent at a frequency of about 1 Hz. These results indicate that the development of cramps involves spinal pathways, although the origin may be peripheral.

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