ABSTRACT: Experimental methods involving painful electrical stimulation of a peripheral nerve showed the existence of a minimum stimulation frequency capable of inducing cramp, termed "threshold frequency" (TF). Our aim was to test an alternative method to induce fasciculations and cramps electrically. Two daily sessions of electrical stimulation of the abductor hallucis muscle were performed in 19 volunteers on 3 days: stimulation trains of 150 monophasic square pulses (duration 152 μs) of increasing frequency (current intensity 30% higher than maximal; frequency of the first trial, 4 pps; recovery between trials, 1 min) were delivered to the main muscle motor point until a cramp developed. Once a cramp was induced the protocol was repeated after 30 min. To verify by electromyography that cramp occurred, a surface electrode array was placed between the motor point and the distal tendon. Ambient and skin temperature were kept constant in all sessions. Fasciculations and cramps were elicited in all subjects. We observed the following median (interquartile range) values of TF: day 1 (session 1), 13 (6) pps; day 1 (session 2), 16 (4) pps; day 2 (session 1), 16 (6) pps; day 2 (session 2), 18 (6) pps; day 3 (session 1), 17 (4) pps; day 3 (session 2), 18 (8) pps. TF intersession intraclass correlation coefficients were 0.82, 0.92, and 0.90 for days 1, 2, and 3, respectively. TF interday intraclass correlation coefficient was 0.85. The absence of pain due to the stimulation and the demonstration of TF reliability support the use of our method for the study of involuntary muscle phenomena.
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RELIABILITY OF A NOVEL NEUROSTIMULATION METHOD TO STUDY INVOLUNTARY MUSCLE PHENOMENA

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Muscle cramp is a sudden, painful, and involuntary contraction of a muscle or part of it. It has a neurogenic origin, as indicated by its electrophysiological definition: periodic bursts of high-frequency (up to 150 Hz) discharges of motor unit action potentials (MUAPs).[25] The number of motor units activated and their discharge frequency increase gradually during the cramp and then subside gradually, with an irregular firing pattern, toward the end.[25] Muscle cramps are very common among healthy people and may also appear as a consequence of lower motoneuron disorders, endocrine and metabolic disorders, and certain medications. Similarly, in both healthy subjects and patients with various neuromuscular disorders, abnormal spontaneous activity of groups of muscle fibers can be observed clinically as fasciculation. Fasciculation is associated with the occurrence of a fasciculation potential, that is, an action potential that has the same configuration as an MUAP but occurs spontaneously.[10] Fasciculations and cramps can be encountered together: electromyography (EMG) during cramp often demonstrates fasciculation potentials both at the beginning and end of the cramp.[25]

The physiological mechanisms of both fasciculations and cramps and their sites of origin are controversial. The central origin hypothesis supposed their origination at the motoneuron level.[1,2,26,28,29,31,36] Of particular relevance, Baldissera et

Abbreviations: ANOVA, analysis of variance; ARV, average rectified value; EMG, electromyography; ICC, intraclass correlation coefficient; MUAP, motor unit action potential; ρ_0, minimally acceptable level of reliability; ρ_1, expected level of reliability; TF, threshold frequency; VAS, visual analog scale
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al.[1,2] proposed that a "bistability" of the motoneuron membrane may be at the origin of the cramp discharge and Obi et al.[29] showed that when the ulnar nerve was blocked with lidocaine at the elbow, no cramp was induced despite the application of high-frequency electrical stimulation at the wrist.

By contrast, Lambert[20] and Bertolasi et al.[4] found that cramps of the flexor hallucis brevis muscle could be induced even after peripheral nerve block by electrical stimulation distal to the block. Moreover, the demonstration that cramps can slowly change in position (a few centimeters per second) over the muscle[30] pointed toward the intramuscular terminal branches of motor axons as their source sites. The same peripheral origin for fasciculations has also been postulated.[11,21] A mechanical deformation of intramuscular nerve terminals caused by muscle contraction may be the main peripheral causal factor of cramp initiation and maintenance.[21] Furthermore, Serrao et al.[32] recently showed that pain induced by injection of hypertonic saline facilitates the occurrence of electrically induced muscle cramps, thus suggesting that thin nociceptive fibers play an important role in the genesis of muscle cramps.

As recently underlined by Miller and Layzer,[25] our understanding of cramps has not progressed significantly in the last decade, mainly due to the lack of experimental models in laboratory animals and experimental methods that would permit objective evaluation of pharmacotherapy. A few experimental methods to induce a cramp of the flexor hallucis brevis muscle by means of repetitive electrical stimulation of a peripheral nerve have been reported: the stimulation frequency was the determinant of whether cramp was induced and the minimum stimulation frequency capable of inducing cramp was termed "threshold frequency" (TF).[4,20,32,34] Stone et al.[34] studied the reliability of the cramp TF by recording two sessions on three separate days; they demonstrated excellent intrasession [intraclass correlation coefficients (ICCs) 0.84, 0.95, 0.98] and intersession (ICC 0.96) reliability. However, the reduced tolerability due to the direct stimulation of the posterior tibial nerve at the ankle limited the applicability of these methods to the study of both patients and healthy populations.

In recent years, interesting insights into the pathophysiology of involuntary muscle phenomena have come from the application of multichannel surface EMG techniques to the study of spatiotemporal development of both fasciculations[11] and cramps.[30] However, in these preliminary experiments the occurrence of the involuntary muscle phenomena was not controlled by the experimenters: one patient with amyotrophic lateral sclerosis who had abundant fasciculations was studied in the former study,[11] whereas cramp-prone subjects able to voluntarily induce cramp in the triceps surae were evaluated in the latter study.[30]

The purpose of our study was to test a method to induce electrically fasciculations and muscle cramps that was more tolerable with respect to the previously described neurostimulation methods and suitable to be used concomitantly with multichannel surface EMG for electrophysiological characterization of fasciculations and cramps.

MATERIALS AND METHODS

Subjects. Nineteen men [median (interquartile range): age 22 (7) years; height 178 (8) cm; weight 75 (15) kg] volunteered to participate in the study. No subject had any known neuromuscular or skeletal impairment. They were asked to refrain from performing any strenuous physical activity for 24 h before each measurement. Each subject 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.

Experimental Protocol. Each subject reported to the laboratory on three separate days. Two cramps of the abductor hallucis muscle of the dominant foot were induced on each of the three days with the following protocol: (1) identification of the main muscle motor point (maximal mechanical response with the minimum current injected) by using a pen electrode (small size cathode: 1 cm^2 surface; Globus Italia, Codognè, Italy), as described by Mandrile et al.,[23] and placement of an adhesive stimulation electrode; (2) placement of an adhesive surface electrode array for EMG detection; (3) identification of the maximal current intensity on the basis of M-wave peak amplitude, as previously described[12]; the stimulation intensity was increased until the M-wave peak amplitude reached a plateau. It was often observed that, after such a plateau and with much larger stimulation current, M-wave amplitude could increase. The maximal current was identified as the current intensity leading to the first rapid increase of M-wave amplitude, followed by an absence of changes for an increase of >10 mA. Three successive trials were performed and the maximal value measured was assumed as the reference; (4) delivery of a first train of electrical pulses (150 stimuli; current intensity

30% supramaximal; stimulation frequency of the first trial: 4 pps); (5) after 1 min of rest, an increase of the stimulation frequency by 2 pps and delivery of a second train of 150 electrical pulses, and so on until a cramp developed, as determined by subject feedback, clinical observation of continuous muscle contraction that persisted after cessation of the stimulation, and presence of involuntary EMG activity after cessation of the stimulation (visual assessment of EMG signal). We applied 150 constant-current pulses of the same intensity (30% supramaximal) in all trials. Once a cramp was induced, 30-min rest was provided to the subject before the stimulation procedure (starting from the frequency of 4 pps and with subsequent increases by 2 pps, until cramp development) was repeated. Thus, six cramps were induced in each subject on the 3 days.

A 0–10 visual analog scale (VAS; 0 represented "no pain" and 10 represented "intolerable intense pain")[18] was used to quantify pain during each trial of electrical stimulation.

Surface EMG Recording, Stimulation Technique, and Temperature Recording. Subjects were comfortably recumbent in a supine position, with plantarflexion of the ankle and inversion of the foot (Fig. 1). Surface EMG signals were detected with a linear adhesive array of eight electrodes (1 mm thick, 3 mm long, and 5 mm apart, LISiN, Turin, Italy; Spes Medica, Battipaglia, Italy) in single-differential configuration. Duration of EMG recording varied between the different trials and consisted of a 5-s prestimulation EMG, EMG (M-wave and involuntary spontaneous activity) recording for the entire stimulation period, and 60-s poststimulation EMG: the subject was asked to keep the muscle relaxed both before and after the stimulation period, to report immediately when he felt a muscle cramp, and to let the cramp develop until it disappeared spontaneously. The linear array was located between the selected motor point and the distal tendon and was aligned in the direction of the longitudinal axis of the muscle. Due to intersubject variability in the pennation architecture of the muscle,[22] we chose this alignment in order to increase the studied portion of the muscle: in fact, the inclination of the fibers with respect to the detection system implied that different channels detected different portions of the muscle.

The region where the optimal location for the array was identified was treated with abrasive paste (Every; Spes Medica). To assure proper electrode–skin contact, 20 μl of conductive gel was inserted into the electrode cavities of the array with a gel

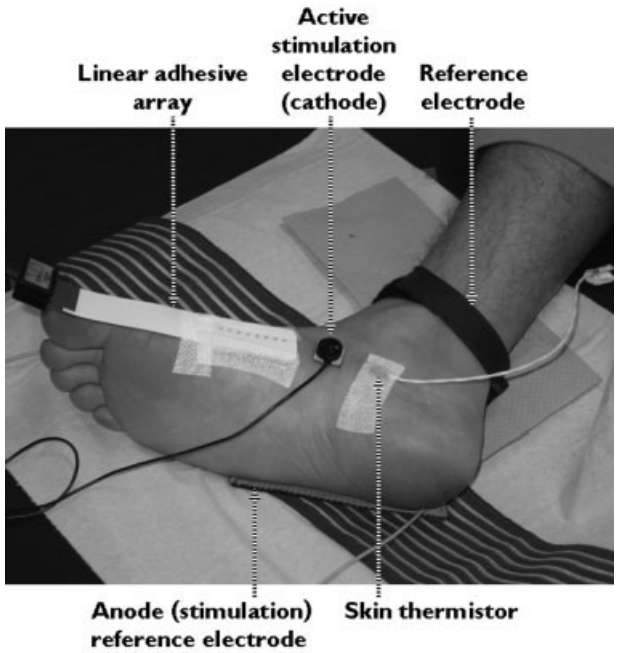

FIGURE 1. Electrode placement on the abductor hallucis muscle. The linear adhesive array used for EMG detection was located between the main muscle motor point, where the active stimulation electrode (cathode) was placed, and the distal tendon. The positions of the anode (stimulation) reference electrode used to close the current loop, of the reference electrode, and of the skin thermistor are also shown.

dispenser (Multipette Plus; Eppendorf AG, Hamburg, Germany). The reference electrode was placed at the ankle. The EMG signals were amplified (EMG16; 16 channel amplifier, LISiN), bandpass-filtered (3-dB bandwidth, 10–500 Hz), sampled at 2,048 samples/s per channel, converted to digital data by a 12-bit acquisition board (National Instruments, Austin, Texas), displayed in real time, and stored on the disk of a personal computer.

Stimulation was provided by a programmable multichannel neuromuscular stimulator (LISiN) equipped with a hybrid output stage to combine the features of a constant-current stimulator during the stimulus with the benefit of a closed-loop constant-voltage output after the stimulus (which quickly drives to zero the voltage on the load with low output impedance, allowing discharge of the electrode–skin capacitances to reduce artifact amplitude and duration). The stimulation waveform was a monophasic square wave of 152 μs duration. The adhesive stimulation electrode (cathode, 35 $\times$ 45 mm, reduced in dimensions to 10 $\times$ 10 mm for the purposes of the study; Spes Medica) was placed over the main muscle motor point while a large electrode (anode, 50 $\times$ 80 mm) was placed over the lateral side of foot to close

Table 1. Stimulation current intensity and threshold frequency (TF) of induced muscle cramp in each of two sessions (1 and 2) on three different days.

		TF Day 1		TF Day 2		TF Day 3	
Subject	Current Intensity (mA)	Session 1 (pps)	Session 2 (pps)	Session 1 (pps)	Session 2 (pps)	Session 1 (pps)	Session 2 (pps)
1	39	10	10	10	12	10	12
2	37	8	10	10	14	8	14
3	43	20	20	22	22	22	22
4	39	16	22	24	26	20	20
5	26	12	14	12	14	14	16
6	26	14	18	16	18	16	18
7	31	10	14	12	16	14	18
8	26	14	14	16	18	20	22
9	20	16	20	18	20	14	14
10	26	18	20	24	26	18	20
11	26	12	16	12	14	18	22
12	26	8	12	14	18	10	14
13	28	12	16	16	20	22	22
14	28	16	18	16	18	18	22
15	28	10	10	10	12	8	10
16	32	16	18	16	18	18	20
17	32	16	16	20	20	18	18
18	16	12	14	16	16	14	14
Median (interquartile range)		13 (6)	16 (4)	16 (6)	18 (6)	17 (4)	18 (8)

the stimulation current loop (monopolar stimulation). Table 1 lists the stimulation current intensity for each subject in all six experimental sessions: it ranged between 16 mA and 43 mA.

After the entire set of contractions of one day (two sessions), the stimulation and detection electrodes were removed and their locations over the skin were measured on the basis of anatomical landmarks to assure comparable electrode positioning on all days.

Skin temperature was continuously recorded during each trial, next to the stimulating electrode (between the stimulation electrode and the proximal tendon), using a skin thermistor (sensitivity ± 0.1°C; Omega Engineering, Stamford, Connecticut).

Data Management and Statistical Analysis. The surface EMG variable of interest was the average rectified value (ARV): it was estimated for each available triplet over the first second (1-s long signal epoch) after the last M-wave (after the end of the stimulation train). A triplet was defined as a group of three single-differential signals provided by adjacent electrodes (five triplets in total provided by the eight-electrode array). Poststimulation ARV estimates for each available triplet were computed as the average of the ARV estimates of the three single-differential signals in the triplet and the highest value among all triplets was chosen as a reference (post-ARV).

Prestimulation ARV estimate (pre-ARV) was computed using the same triplet, over a 1-s long signal epoch, before the occurrence of the first M-wave elicited by the stimulation train.

To verify by EMG that cramp occurred, we calculated for each trial of each session the ratio between post-ARV and pre-ARV; thereafter, the extreme Studentized deviate method or Grubbs' test[3] was applied to all ARV ratios of the session to identify the TF value. As represented in Figure 2, the ARV ratio detected during the cramp was significantly higher than the ARV ratios calculated for subthreshold trials: this allowed identifying it as an outlier with respect to the other values in the session.

TF intersession and interday reliability were assessed using intraclass correlation coefficients (ICCs [3,1]).[33] A sample size of 19 subjects for analysis of TF reliability was established using the approximate method developed by Walter et al.,[35] based on $\alpha = 0.05$ and $\beta = 0.20$, indicating an expected level of reliability (ρ_1) of 0.90,[34] and a minimally acceptable level of reliability (ρ_0) of 0.70. The number of replicates per subject (n) was set to 6.

Fasciculation frequency (expressed as number/min) was calculated for each trial after identification

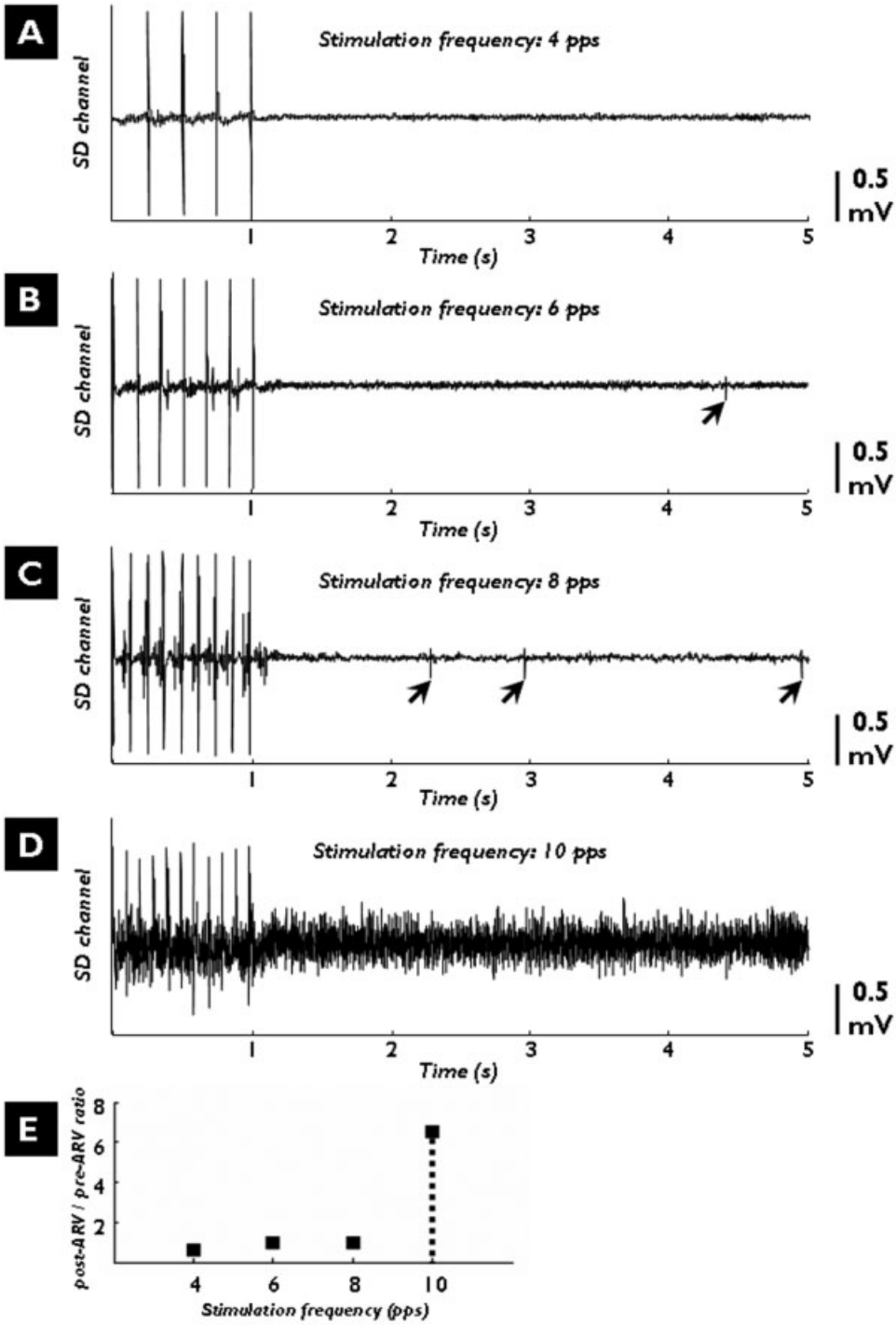

FIGURE 2. (A–D) Single-channel EMG recordings in different trials of the same experimental session from one subject. The last second of the stimulation train (few M-waves) and the first 5 s of the poststimulation EMG recording are shown. No EMG activity is evident for the first trial **(A)**. One fasciculation potential (arrow) is evident for the second trial **(B)**. Three fasciculation potentials (arrows) are evident for the third trial **(C)**. Continuous muscle activity that began during the stimulation and persisted after cessation of the stimulus is evident for the fourth trial **(D)**. **(E)** Values of ratio between post-ARV and pre-ARV in the different trials from the same subject of the other panels. The ARV ratio detected for the cramp trial (dotted line) was significantly higher (Grubbs' test, $P < 0.05$) than the ARV ratios calculated for subthreshold trials.

of fasciculation potentials by visual inspection of the poststimulation EMG recordings.

The nonparametric Wilcoxon paired test was adopted to compare both the fasciculation frequency and VAS ratings between the first trial and the next-to-last trial as well as to compare the TF values of the two sessions on each of the three days. Friedman's analysis of variance (ANOVA) followed by Dunn's post-hoc test were used for comparing both the TF values and VAS ratings of the three days as well as in the comparison of the skin temperature values across and among sessions. The Spearman test was applied to assess the correlation both between subthreshold fasciculation frequency (counted in the next-to-last trial) and TF value and between VAS ratings of pain and current amplitude. Statistical analysis was performed with SPSS 13.0 for Windows (SPSS Inc., Chicago, Illinois) software package. All values are given as median (interquartile range).

RESULTS

Cramp Comparison between Sessions and between Days. We were able to elicit two cramps per day for all three days in 18 subjects. In total, 108 muscle cramps were recorded in the abductor hallucis muscle of subjects: in all cases the three different criteria for cramp identification (subject feedback, clinical evidence, significance of the Grubbs' test) were satisfied. Figure 3 shows three examples of spatiotemporal development of cramp: in Figure 3A the cramp developed in a large muscle area, then decreased in size and intensity until it disappeared; in Figure 3C the cramp either developed in a small area and then changed the position of its focus after 10 s, or quickly disappeared and a second cramp developed after a large fasciculation; in Figure 3E the cramp involved a small portion of the muscle and did not shift its location. The three examples were representative, respectively, of 60%, 5%, and 35% of the cramps we observed.

In 15 of 18 subjects post-ARV (the highest value among all triplets) was calculated for the same triplet in all six sessions: this means that, in almost all cases, the beginning of the cramp always involved the same portion of the muscle.

One subject was excluded from the sample because a cramp could not be elicited during the sessions of the third day (we stopped the experiment after reaching a stimulation frequency of 50 pps, a value double that of the previous 2 days), but only fasciculation potentials were observed (Fig. 4).

Analysis of Threshold Frequency Reliability. Table 1 lists the TF values for each of the 18 subjects. The median (interquartile range) values of TF for the different days and sessions are shown. Significant differences where observed on all days between the TF values of the two sessions: day 1 ($P < 0.01$), day 2 ($P < 0.001$), and day 3 ($P < 0.01$). Friedman's ANOVA and Dunn's test showed significant differences ($P < 0.001$) in TF values both between day 1 and day 2 and between day 1 and day 3, whereas no significant difference was found between day 2 and day 3. TF intersession ICCs (reliability) were 0.82, 0.92, and 0.90 for days 1, 2, and 3, respectively. The TF interday ICC was 0.85.

Analysis of Fasciculation Potentials. We were able to elicit fasciculation potentials in all sessions of each day (in the entire group of 19 subjects). In all cases the fasciculation frequency increased significantly between the first and next-to-last trial (Table 2), as in the example given in Figure 2.

No significant correlation was observed between subthreshold fasciculation frequency (counted in the next-to-last trial) and TF value ($r = -0.04$, $P = 0.72$). No colocalization was observed between cramps (the 35% of cramps that involved a small portion of the muscle) and fasciculations: fasciculation potentials were evident in almost all channels of the array, independently of the subsequent location of the cramp.

VAS Ratings of Pain during Electrical Stimulation. No subject reported pain or discomfort due to the stimulation. VAS ratings of pain were as follows (median values for the two sessions of each day are reported): day 1 (first trial), 1.5 (1.2); day 1 (next-to-last trial), 1.7 (2.6); day 2 (first trial), 1.0 (1.5); day 2 (next-to-last trial), 1.1 (0.9); day 3 (first trial), 1.0 (0.4); day 3 (next-to-last trial), 1.1 (0.4). Significant differences ($P < 0.01$) were observed on all three days between the first trial and the next-to-last trial. Significant differences were also observed both between day 1 and day 2 ($P < 0.01$) and between day 1 and day 3 ($P < 0.001$). No significant correlation was found in each of the three days between VAS ratings of pain and amplitude of the stimulation current.

Skin Temperature Recordings. Skin temperature remained constant across the different trials of each session and no significant differences were observed between the six sessions.

DISCUSSION

Cramp and Fasciculation Induction by Transcutaneous Stimulation. The present study shows that cramps and fasciculations can easily be obtained and analyzed using the proposed neurostimulation method and multichannel surface EMG.

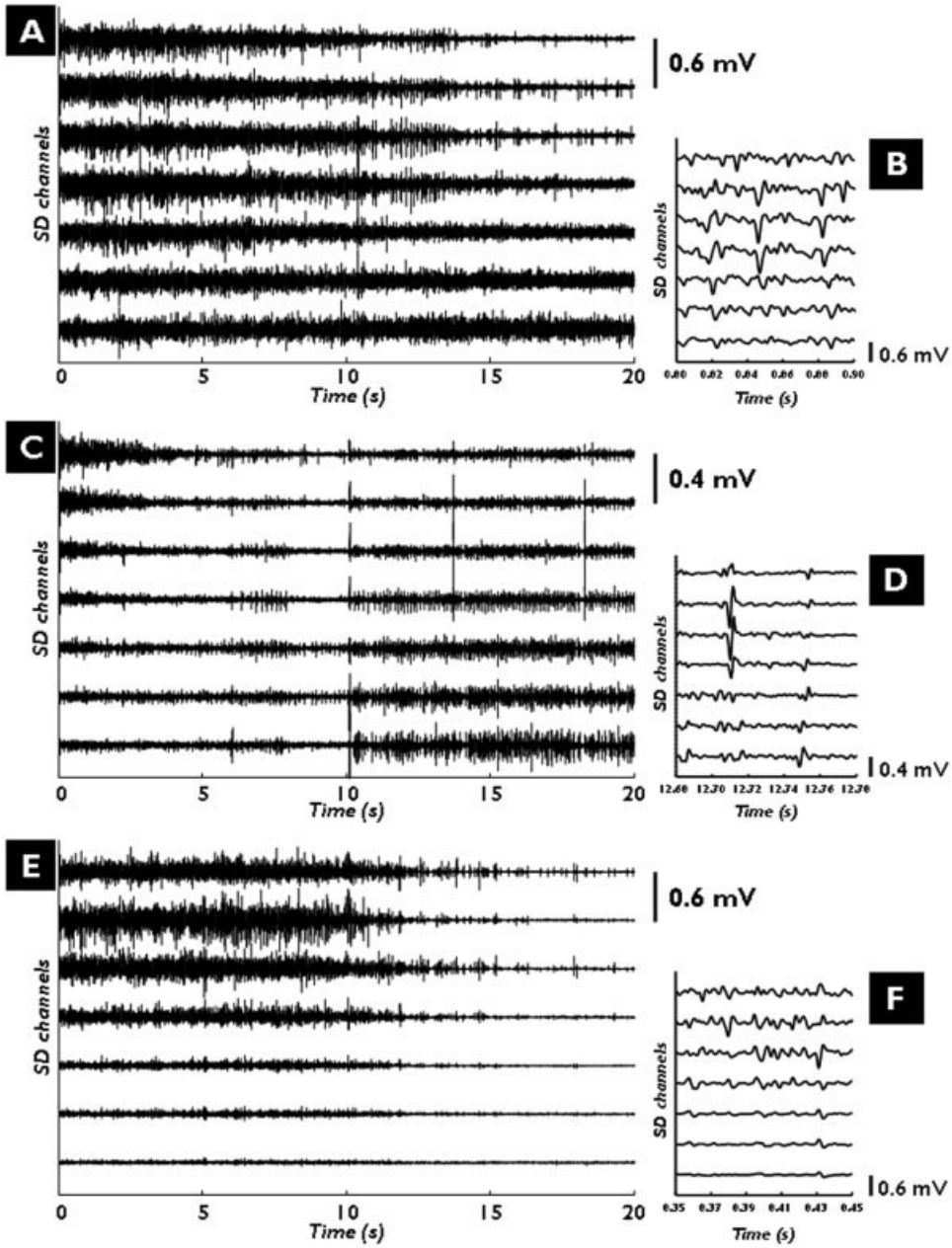

FIGURE 3. Examples of poststimulation EMG recording in three different muscle cramps. **(A)** The cramp developed in a large muscle area, then decreased in size and intensity. **(B)** Zoomed portion of the signal during the first second of the cramp. **(C)** The cramp either developed in a small area and then changed the position of its focus after 10 s, or quickly disappeared and a second cramp developed after a large fasciculation. **(D)** Zoomed portion of the signal during the 12th second of the cramp. **(E)** The cramp involved a small portion of the muscle and disappeared after about 10 s. **(F)** Zoomed portion of the signal during the 8th second of the cramp.

With respect to other laboratory methods of cramp induction by means of either voluntary contraction of a muscle[30] or performing a fatiguing exercise protocol,[17] our neurostimulation method was more effective (and reproducible) since it triggered the same cramp of the muscle under study in all subjects, whereas this is not always observed in the cases of contraction- or fatiguing exercise-elicited muscle cramps.

With respect to the previously reported methods of electrical induction of muscle cramps,[4,20,32,34] our stimulation of the main muscle motor point has the considerable advantage of triggering a cramp in the muscle (or in a part of it) without the pain due to

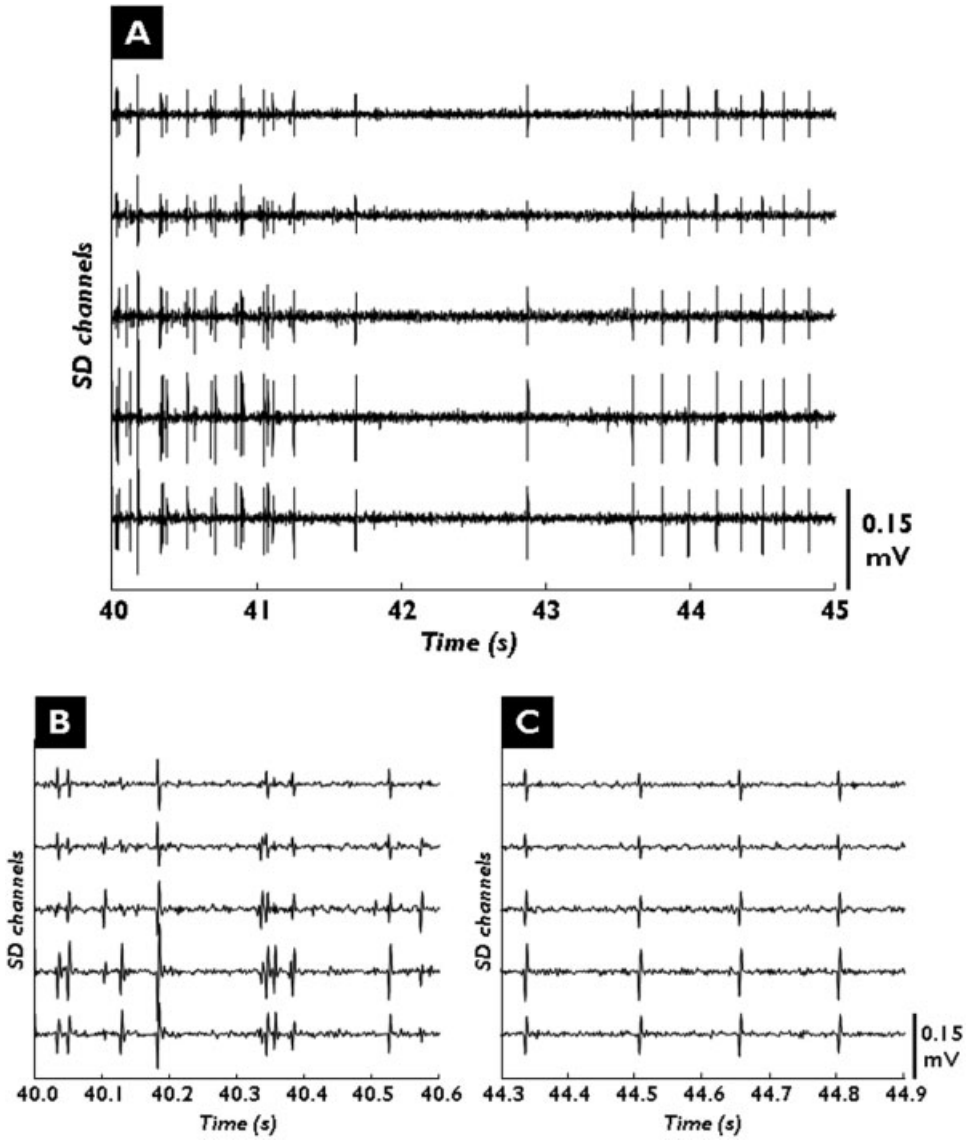

FIGURE 4. (A) Example of fasciculation potentials in the poststimulation EMG recording (between 40 s and 45 s from the end of the stimulation train; frequency: 50 pps) from one subject. **(B,C)** Two zoomed portions of the signal: 10 fasciculation potentials are visible in the initial 0.6 s: on the basis of shape differences they could be assigned to different electrical sources. Four fasciculation potentials with a similar waveform were recorded between 44.3 s and 44.9 s.

stimulation of the sensitive afferent fibers of the peripheral nerve trunk. Moreover, the main muscle motor point can easily be identified: it was consistently found just inferior and posterior to the navicular tuberosity, in keeping with a previous study.[9] As expected, electrical stimulation of the muscle motor

Table 2. Median (interquartile range) values of fasciculation frequency in each of two sessions (1 and 2) on three different days.

	Fasciculation frequency Session 1			Fasciculation frequency Session 2		
	First trial (no./min)	Next-to-last trial (no./min)	*P*-value	First trial (no./min)	Next-to-last trial (no./min)	*P*-value
Day 1	8.2 (13.0)	15.8 (16.5)	<0.05	8.2 (13.6)	18.0 (7.5)	<0.05
Day 2	6.8 (13.6)	16.6 (17.4)	<0.01	8.3 (9.3)	16.6 (11.4)	<0.05
Day 3	9.5 (8.2)	22 (18)	<0.001	6.8 (10.9)	15.0 (25.2)	0.001

point required different current amplitudes between different subjects. Even if the abductor hallucis motor point can easily be found in a specific portion of the muscle, interindividual variability exists in the route of motor axons: the deeper the motor axons and their terminal branches, the greater is the current amplitude needed for supramaximal stimulation.

Consistent with previous reports,[4,20,32,34] in our experimental conditions the decisive element for cramp (and fasciculation) induction appeared to be the stimulation frequency: for each subject a critical stimulation frequency, below which cramps were not elicited, was identified. The physiological determinants of the association between the frequency of the delivered stimulation train and the trigger of an autonomous and repetitive excitation of the terminal branches of motor axons are not understood. Moreover, in the present series the threshold frequency tended to be lower in cramp-prone subjects (identified on the basis of anamnestic data of cramp occurrence both at rest and during or after exercise): this could mean that the threshold frequency may estimate the individual susceptibility to cramp development. This observation, previously reported by Bertolasi et al.,[4] awaits explanation.

Interestingly, in some of our cases a cramp was triggered before the end of the stimulation train, indicating that a shorter stimulation duration (and therefore a smaller number of stimuli) was sufficient to initiate it. The number of stimuli needed to trigger a cramp and the minimal stimulation duration required remain to be investigated.

Analysis of Threshold Frequency Reliability. The reliability of our neurostimulation method was demonstrated not only by reliability analysis applied to the TF, but also by considering the EMG finding of consistency in cramp localization over the first second after the end of stimulation. The reliability of measurement of a given parameter is inversely related to the variability shown within a set of measurements of that parameter observed in the same subject under similar conditions. We standardized the measurement conditions to avoid possible biases: in all sessions, ambient and skin temperature was kept constant and the foot was kept in the same position.

As observed by Stone et al.,[34] in our study the main cause of TF variability both between sessions and between days was subjects' accommodation to the stimulus: in fact, the improved reliability coefficients over time demonstrated the existence of a learning effect. Accommodation between consecutive sessions and between different days was also proved by subjects' sensation. The physiological mechanisms underlying the short-term decrease in axonal excitability (which occurred between consecutive sessions) and the long-lasting adaptations (observed between different days) are thought to be different. The short-term excitability decrease we observed is similar to both the activity-dependent decrease and the posttetanic decrease in excitability of motor axons after either voluntary activation or prolonged repetitive stimulation.[5,6,19,27] These decreases are a function of an axonal hyperpolarization, which results from activation of the electrogenic Na^+-K^+ pump to correct the intracellular Na^+ accumulation that occurs when an axon conducts trains of impulses.[6,19,27] Accommodation between different days could be viewed as a form of activity-dependent functional plasticity of the peripheral nervous system. As demonstrated by Debanne et al.,[8] the axon should not only be considered as a simple structure that reliably transmits the action potential from the cell body to the nerve terminal: it is also able to express nonsynaptic mechanisms of plasticity, which range from activation and inactivation[7] to dynamic compartmentalization[15,16] of ionic channels. This nonsynaptic form of plasticity could underlie the variability in TF that we observed between different days (mainly between the first day and the subsequent 2 days) and should always be considered in the planning of experimental protocols with serial measurements on the same subject. An initial orientation session should always be included, with the objective to allow the subject to experience both electrical stimulation and fasciculation and cramp induction.

Spatiotemporal Development of Cramp. Threshold stimulation frequencies triggered muscle cramp events that showed three main patterns of spatiotemporal development. Unlike Roeleveld et al.,[30] who observed a slow change in position of the cramp focus in about 50% of the cramps and some cases of multifocal cramp, in our series the focus in 95% of cramps did not change in position. This discrepancy could be the result of our low accuracy in the study of cramp distribution due to the 7-channel recordings we adopted: a high-density surface EMG technique is needed not only to examine the spatiotemporal characteristics of MUAPs during cramp but also to distinguish between spreading and multifocal cramps. Moreover, intercramp biological variability could account for the discrepancy: above-threshold stimulation frequencies could be necessary to reproduce the number of motor units involved and observed during voluntarily induced cramps. Finally, it

must be pointed out that we studied a muscle that is very different with respect to the muscle studied by Roeleveld et al.[30] (triceps surae) in both size and motor unit composition: cramp behavior could be different in different muscles.

Visual inspection of EMG recordings during cramp showed high-frequency bursts of propagating MUAPs (presenting low propagation velocity, shape changes during propagation, and variable propagation paths), and nonpropagating potentials. The characteristics of propagating MUAPs and the nonpropagating signal components shown in Figure 3B,D,F could be related to a number of factors, such as (1) lack of alignment between the muscle fibers and the detection system[13]; (2) scattered projection of endplates and distal tendon along the recording electrodes (resulting from the inclination of the muscle fibers)[24]; and (3) extinction of the action potential at the tendon (end-of-fiber effect).[14]

Analysis of Fasciculation Potentials. We are not aware of previous studies that investigated the relationship between fasciculation frequency and frequency of the electrical stimulation needed for cramp induction. Our data show that stimulation frequencies below (cramp) threshold can change axonal excitability, as confirmed by the significant differences in fasciculation frequency that were observed in all sessions (between the first and next-to-last trial) and that change in nerve excitability is a necessary, but not sufficient, condition for cramp induction, as confirmed by the lack of correlation between the subthreshold fasciculation frequency and cramp threshold frequency as well as by the absence of colocalization between subthreshold fasciculations and cramp. This finding is in keeping with the clinical evidence that most patients with benign fasciculations do not have frequent cramps and, similarly, patients with frequent muscle cramps may not have frequent fasciculations.[25] However, we cannot exclude the possibility that the observed absence of correlation was the result of our underestimation of the fasciculation frequency: in fact, Drost et al.[11] showed that when surface EMG signals are recorded with electrodes in a bipolar configuration, lower fasciculation potentials can be identified with respect to the monopolar electrode configuration, since the monopolar montage has a greater detection volume, and hence records larger numbers of fasciculation potentials than the bipolar configuration.

Limitations and Clinical Implications. This study concerns the abductor hallucis, a muscle in which a cramp can be induced easily and reliably. This muscle is not usually electrically stimulated for reasons other than cramp research. Other muscles are either of greater interest in rehabilitation or subject to stimulation as a countermeasure in microgravity environments. If and how cramps may be an undesired result of stimulation of these muscles is unknown and should be the topic of further research. Furthermore, we examined a population of young and healthy male subjects: future studies are needed to evaluate whether the proposed neurostimulation method is tolerable and reproducible also in other populations of older subjects or those with underlying pathological processes.

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