Motor unit behavior during clonus

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ABSTRACT

Medial gastrocnemius surface electromyographic activity (EMG) and intramuscular EMG were recorded from six individuals with chronic cervical spinal cord injury to document the recruitment order of motor units during clonus. Four subjects induced clonus that lasted up to 30 seconds while two subjects induced clonus that they actively stopped after one minute. Mean clonus frequency in different subjects ranged from 4.7-7.0 Hz. Most of the 166 motor units recorded during clonus (98 %) fired once during each contraction but at slightly different times during each cycle. Other motor units fired during some clonus cycles (1 %) or in bursts (1 %). When 59 pairs of units were monitored over consecutive clonus cycles (n=5-89 cycles), only 8 pairs of units altered their recruitment order in some cycles. Recruitment reversals only occurred in units that fired close together in the clonus cycle. These data demonstrate that orderly motor unit recruitment occurs during involuntary contractions of muscles paralyzed chronically by cervical spinal cord injury, providing further support for the importance of spinal mechanisms in the control of human motor unit behavior.

Keywords: motor unit recruitment, human spinal cord injury, spasticity, muscle spasm
INTRODUCTION

A few weeks after spinal cord injury, paralyzed triceps surae muscles often contract rhythmically in response to peripheral stimuli to produce repetitive, brisk (≈ 5 Hz) ankle dorsiflexion and plantar flexion. Known as clonus, these repetitive contractions may persist for just a few seconds or for several minutes and can disrupt the daily lives of spinal cord injured people (11, 27). Muscle and motor unit activity during clonus must reflect spinal and reflex mechanisms when the spinal cord injury interrupts voluntary control of the triceps motoneuron pool (45). Consequently some investigators suggest that peripheral inputs activate central structures to produce the repetitive contractions that characterize clonus (4, 14, 48, 49), whereas others consider these involuntary contractions involve repeated activation of the stretch reflex (37, 38).

Stretch of triceps surae muscles is the same stimulus that was used to excite motoneurons in the studies that described the size-ordered recruitment of motoneurons in acutely decerebrate cat preparations (21). Since the formulation of the size principle, ordered motoneuron recruitment has been shown to occur in many animal muscles that have been excited by specific afferent or descending inputs (10, 21, 25, 40, 43, 52). This same principle operates during reflex, isometric and ballistic voluntary contractions of various human muscles (7, 13, 33). It also applies in some studies involving dynamic contractions (39, 41, 46), although the prevalence of selective recruitment of high threshold motor units during dynamic lengthening contractions is controversial (22, 35). Thus, evidence from either acutely reduced animal preparations or from healthy human muscles suggests that spinal mechanisms (the intrinsic properties of motoneurons and their response to synaptic inputs) dominate the orderly recruitment of motoneurons (12).

The aim of the present study was to test the hypothesis that motor unit recruitment was ordered during clonus in medial gastrocnemii muscles of individuals with chronic cervical spinal cord injury. The repeated contractions of paralyzed human muscles during clonus provide a unique opportunity to evaluate whether spinal mechanisms dictate the recruitment order of motor units during involuntary contractions because the ability of the subjects to voluntarily control these muscles has been disrupted by the injury. A description of the recruitment order of lumbar motoneurons during clonus can also reveal whether one of
the basic mechanisms used to generate force in muscles is preserved after the neuromuscular adaptations that occur following chronic paralysis.
METHODS

Subjects. One woman and five men (mean age: 37 years (SD5; range: 33-51 years) with chronic cervical spinal cord injury (mean time since injury: 12 years (SD3); range: 6-18 years) were evaluated. The injuries were caused by motor vehicle accidents (n=3), diving mishaps (n=2) or by a fall (n=1). The current injury levels were at C5 (n=1), C6 (n=3) or C7 (n=2), as assessed by the American Spinal Injury Association (ASIA; 30), and were complete in 5 subjects (ASIA A) and incomplete in 1 subject (ASIA B). The subjects had no voluntary control of any leg muscles, judged by an inability to generate EMG or to produce any palpable muscle shortening during repeated attempts to contract these muscles. Thus, the clonus that each subject triggered in their leg muscles involved only involuntary muscle contractions. The subjects took no medication to dampen muscle spasms and the spinal cord injury was their only known neurological disorder. The Institutional Review Board of the University of Miami approved this study. Each subject gave informed, written consent before participating in the experiments. One subject returned to the laboratory on a different day and repeated the experiment to establish the consistency of the self-induced clonus.

Procedure. Each subject sat in their wheelchair with the foot of the test leg (that in which clonus was triggered most easily) placed on a platform such that the hip, knee, and ankle joints were at approximately 90°, 120°, and 90°, respectively. The dorsum of the foot was held in place with a Velcro strap (Fig. 1A). A pair of self-adhesive gel electrodes (Medtronic Andover Medical Inc., Haverhill, MA) was attached to the skin over the medial gastrocnemius muscle to record surface electromyographic activity (EMG). One electrode was positioned on the middle of the muscle belly while the other electrode was 5 cm distal. A monopolar needle electrode (0.2 mm diameter insulated tungsten shaft with a 10-15 µm exposed tip, impedance 200-400 kΩ; 19) was inserted into medial gastrocnemius to record single motor unit potentials. A transducer (Model FT10, force range: 0.5g-10 kg; Astro-Med, Inc., West Warwick, RI) held in a wooden constraint was placed beneath the foot platform to record the sum of the forces generated by the muscles active during clonus. Surface EMG, intramuscular EMG and force signals were amplified,
filtered (30-1000 Hz, 100-10,000 Hz, DC-100 Hz,) and sampled on-line (3,200 Hz, 12,800 Hz and 400 Hz, respectively) to a computer using a SC/Zoom system (Physiology Section, Umeå University, Sweden).

Protocol. Each subject manually induced clonus four to eight times (mean: 6, SD2) either by gently lifting, then releasing the knee of the test leg with their hands or by adjusting the way they sat in their chair. If clonus continued for 60 s, the subject stopped the contractions by leaning forward or by putting pressure on the test knee. Subjects had at least 5 minutes rest between spasms involving clonus. During this rest, the needle electrode was repositioned in medial gastrocnemius (on average, 6 times per experiment, SD2). Each recording site involved a new skin penetration at least half an inch from the previous position so it was likely that the potentials recorded during each spasm involving clonus were from different motor units. The intramuscular signals were monitored during clonus by listening for trains of potentials over the loudspeaker output from the amplifier or by observing the potentials displayed on an oscilloscope. If the motor unit signals deteriorated during clonus, the intramuscular electrode was moved slightly within the muscle until another train of unit potentials was heard or seen.

Analysis of cycle timing and intensity. Surface EMG from medial gastrocnemius was rectified and the integral measured at the onset and offset of each burst of EMG (Fig. 1B, dotted and dashed lines respectively). The period of relative EMG silence following the burst of EMG was termed the interburst. Together, one burst of surface EMG and the following interburst constituted one clonus cycle (Fig. 1B). For each spasm involving clonus, the durations of the bursts, interbursts and clonus cycles were calculated from the burst onset and offset times. To compare clonus cycles across spasms and subjects, the cycle time was normalized. The onset of the burst was defined as 0 % of cycle time and the termination of the interburst was 100 % of cycle time. The integrated EMG during each burst was normalized by the integration time to provide the average EMG.
Unit identification and recruitment time. Waveform duration and amplitude criteria set using Zoom software were used to identify potentials from single medial gastrocnemius motor units recorded in the intramuscular EMG (15). The potentials identified for each single motor unit were overlaid to verify that the classification of the potentials was accurate (Fig. 1B). The time at which each unit fired in different clonus cycles was measured (absolute recruitment time, with each burst onset as 0 ms), and then converted to relative cycle time, termed relative recruitment time. For example, the two units identified in Figure 1B (●, *) fired at 11 ms and 116 ms (absolute recruitment times) into the first clonus cycle (211 ms), which represented relative recruitment times of 5 % and 55 % cycle time. These two units were activated in 85 and 141 cycles, respectively, at average relative recruitment times of 6 % (SD2) and 53 % (SD3) cycle, respectively. To assess whether the variation in the recruitment time of a motor unit across cycles was merely a function of the variation in cycle duration, the coefficients of variations for motor unit firing rate and cycle rate were compared (paired t-test) for all units.

Unit recruitment order. Each clonus cycle was considered a separate event with respect to recruitment of motor units. The recruitment order of pairs of motor units that were coactive in at least five cycles of clonus was monitored by comparing the relative cycle times at which the units fired in different cycles. The unit with the lowest mean relative cycle time or recruitment time was ranked first. Deviations from this recruitment pattern were classified as reversals of recruitment. The incidence of recruitment reversals was expressed as a percentage of the number of cycles in which the pair of units was coactive.
RESULTS

Clonus timing. Clonus in different subjects involved varying numbers of rhythmic involuntary contractions of leg muscles. In three subjects clonus was short, involving from 5 to 22 contractions. Clonus was of intermediate duration in another subject (174 contractions, SD43), but was deliberately stopped by the other two subjects after 60 s (> 300 contractions). Mean clonus frequency varied between 4.7 and 7.0 Hz for different subjects, with the burst of medial gastrocnemius EMG lasting from 25 % to 40 % of the cycle (mean 28 % cycle, SD6).

Clonus was extremely reproducible for any given subject. Figure 2 shows the magnitude of the medial gastrocnemius surface EMG (A-C), burst and cycle duration (D-F) recorded during one spasm (A,D), during six spasms involving clonus from the same experiment (B,E) and during another seven spasms involving clonus from a different experiment on the same subject (C,F). During the first few contractions, there was an increase in EMG and a decline in cycle duration. Thereafter, clonus in this subject involved a characteristic decline in EMG but little change in cycle timing. The same timing and EMG patterns were found for the other subject with clonus of long duration. In the four subjects in which clonus ended spontaneously, the EMG also declined over time but cycle duration increased with time.

Motor unit firing patterns. Data were obtained from 166 motor units, with unitary records involving 5 to 226 potentials (mean: 23 potentials, SD30). Three distinct patterns of unit activity were evident during clonus (Fig. 3). Units either fired one potential every cycle (Fig. 3A), one potential in some cycles (Fig. 3B), or in bursts (Fig. 3C). Motor units with these different firing patterns were recorded throughout the clonus cycle (i.e. in the burst, interburst or both phases of the cycle) and in three subjects. Units fired only once per cycle in the other three subjects.

The majority of units fired once per clonus cycle (98 %), an example of which is shown in Figure 3A during clonus at a mean frequency of 4.7 Hz (SD0.1). A few units (1 %) were active in some, but not
necessarily consecutive clonus cycles. In Figure 3B, one unit (●) fired in only 7 of 9 cycles whereas another unit (●) fired in all 9 cycles. The potentials of the latter unit were reflected in the surface EMG suggesting that the intramuscular recordings were stable and that the other unit was not recruited in two of the cycles. Other units (1 %) fired more than once per cycle, usually with one short interval amongst intervals close to the frequency of the clonus (Fig. 3C).

**Motor unit firing variability.** Mean unit firing rate during clonus was 5.5 Hz (SD0.9; n=166 units). The temporal variability in the recruitment of each unit across different cycles was assessed by comparing the coefficient of variation for unit firing rate to that of cycle rate. Figure 3A shows 4 cycles of clonus in which the cycle durations varied by 15 ms. The active unit (●) shifted its firing time relative to the burst onsets by 23 ms. Over 116 cycles, the mean firing rate of this unit was 4.71 Hz (SD0.18). Its firing variability across these cycles was greater than the variability in the cycle rate (mean coefficients of variation: 3.82 % and 2.24 %, respectively).

Figure 4 compares the instantaneous firing rates of 5 units to the rates of the corresponding clonus cycles. These data were recorded during 2 of the spasms involving clonus shown in Figure 2. The units in A-E fired at an average of 4.79 Hz (SD0.14), 4.79 Hz (SD0.18), 4.79 Hz (SD0.09), 4.80 Hz (SD0.17), and 4.81 Hz (SD0.45) to give mean coefficients of variation of 2.79 %, 3.73 %, 1.95 %, 3.51 % and 9.47 %, respectively. In each case, the variability in unit firing exceeded the variability in the rates of the clonus cycles (mean coefficients of variation for cycle rate: 0.88 %, 0.83 %, 1.74 %, 1.63 %, 1.34 %, respectively).

[Fig. 4 near here]

Figure 5 compares the mean coefficient of variations for unit firing rate and cycle duration for 166 units. Most of the data lie below the unity line indicating that motor unit firing rates varied more that the cycle rates. The mean coefficient of variation for unit firing rate (5.1 %, SD3.9) was significantly greater than the mean coefficient of variation for cycle rate (2.7 %, SD2.1; p<0.001). Thus, the variation in the firing behavior of different units was not merely a consequence of changes in cycle duration.
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Motor unit recruitment order. Recruitment order was compared for 59 motor unit pairs from three different subjects. Pairs of motor units were coactive in 5 to 89 clonus cycles (mean 29 cycles, SD22). Most motor unit pairs (n=51 or 86%) were repeatedly recruited in a fixed order as shown in Figure 6A where the three identified units had a consistent firing sequence throughout 20 clonus cycles. One unit (+) was always recruited near the beginning of the burst (mean relative cycle time: 6%, SD2). Another unit (●) was always recruited near the middle of the burst (mean relative cycle time: 18%, SD3), while the other unit (*) fired in the interburst (mean relative cycle time: 53%, SD3).

Recruitment reversals were seen in only 8 motor unit pairs (14%). These reversals occurred in motor unit pairs that fired close together in the clonus cycle (Fig. 6B). The mean time difference between potentials of motor unit pairs showing reversals was 8.0 ms (SD12.3, range: 0.3-38 ms) compared to 50.7 ms (SD34.6, range: 5.3-127.2 ms) for unit pairs showing no reversals. Even when unit pairs showed reversals, it was a rare occurrence. The reversal percentage for 6 out of the 8 pairs was under 10%. Those unit pairs having more than 10% reversals fired within 5 ms of each other.

Figure 4 also shows that coactive motor units differed their firing variability. The unit shown in Figure 4A had a less variable firing pattern than the unit in Figure 4B and was recruited earlier in the clonus cycle (average recruitment was at 6.1% cycle time (SD2.0) versus 18.5% cycle time (SD2.8), respectively). Similarly, the units in Figures 4C-E were coactive in some cycles of a different spasm involving clonus with average recruitment times of 5.8% cycle time (SD2.0), 17.3% cycle time (SD3.1), and 38.3% cycle time (SD7.0), respectively. Again, the earlier a unit was recruited in the cycle, the less variable its firing behavior. When all unit pairs coactive during the first half of the clonus cycle were considered (n=33 units), the median coefficient of variation for the firing rate of units recruited first (2.6%) was significantly lower than that of the units recruited later (3.1%; p=0.02).
DISCUSSION

The present study shows that 51 of the 59 pairs of medial gastrocnemius motor units recorded during clonus in individuals with chronic cervical spinal cord injury were repeatedly recruited in the same sequence. Eight motor unit pairs occasionally reversed their recruitment order but all these motor units were activated close together in the clonus cycle. Most (98%) of the 166 single motor units studied only fired once per clonus cycle, but the variability in the firing of motor units across cycles was greater than the variability in the durations of the clonus cycles.

Orderly motor unit recruitment. The majority of motor units active during clonus were recruited in a fixed order throughout the clonus cycle, as occurs during stretch reflexes (7) and isometric voluntary contractions (8, 33, 51) of various human muscles with intact innervation. When recruitment reversals did occur, it was only in some cycles and was always between pairs of units that fired close together in the clonus cycles (Fig. 6B). Thus, clonus seems to evoke orderly motor unit recruitment patterns that are similar to those reported in some studies involving dynamic voluntary contractions (39, 41, 46). In other studies, some higher threshold units have been recruited selectively during active lengthening contractions (22, 35). However, these contractions differ from those recorded during clonus in that the medial gastrocnemius muscle undergoes passive lengthening (Fig. 1).

Stereotyped recruitment of motoneurons has been seen in most animal studies in which various excitatory and/or inhibitory inputs to pairs of motoneurons have been activated selectively (12). These studies suggest that the order of motoneuron recruitment is largely determined by intrinsic motoneuron properties that tend to co-vary with motoneuron size (18, 21, 24). The high incidence of orderly motor unit recruitment in human skeletal muscles that, due to spinal trauma, are under no voluntary control from higher centers, suggests that spinal systems also dominate the stereotyped excitation of human motoneurons during clonus. Thus, any changes in spinal neuron excitability, synaptic inputs or muscle properties due to injury (5, 6, 9, 31) were appropriate to preserve an orderly pattern of motor unit recruitment, as found during voluntary contractions of muscles innervated from the level of injury (44).
Orderly motor unit recruitment by size? As clonus involves shortening of various muscles, the twitch force associated with the activation of each single motor unit potential (33) was not a useful measure of motor unit size. Similarly, the potentials from each of the identified pairs of single motor units were not often reflected in the averaged surface EMG, so we could not systematically evaluate whether the recruitment of the motor unit pairs during clonus was ordered by the size of the single motor unit potential (32). However, Figure 7 shows an example of the averaged surface EMG and intramuscular potentials of two motor units detected simultaneously with a selective electrode, making it very likely that their muscle territories overlapped and that the fibers were a similar distance from the surface electrodes. Thus, the averaged surface EMG associated with the firing of these two units should provide a reasonable indication of unit size. The motor unit with the small amplitude surface EMG potential was active near the end of the interburst (average recruitment time: 82 % cycle time, Fig. 7A), so was presumably recruited by the afferent activity from the previous contraction and before the unit with the larger amplitude surface EMG potential that fired during the EMG burst (20 % cycle time; Fig. 7B).

Microneurographic recordings during clonus have shown that muscle spindle activity begins just after the start of muscle relaxation and lasts for about 50 ms (20, 42). In Figure 6A, peak clonus torque occurred at an average of 34 % cycle time, near the end of the burst of EMG. Conduction latency to and from the spinal cord via large diameters axons would be expected to be \( \approx 35 \) ms (28, 47, 50), representing about 20 % of the clonus cycle. Activity from the most excitable motoneurons could therefore be recorded in the muscle during the latter half of the clonus cycle or in the interburst. Provided our assumptions about the magnitude of surface EMG signals and unit excitability are valid, the recordings in Figure 7 suggest that recruitment was size-ordered for these two units during clonus. The reduced firing variability for the first unit recruited in a pair of coactive units (Fig. 4) also suggests that earlier recruited units were closer to their threshold for rhythmic activation (36). However, further studies are needed to provide clear evidence that the orderly recruitment of motor units during clonus is ordered by size.

[Fig. 7 near here]
Variability in motor unit firing across cycles. We have assumed that each clonus cycle is a separate event involving recruitment of various motor units, as shown by the lack of activity in a few units in some cycles of clonus, particularly when the potentials of other units were present in every cycle and were evident in both the surface EMG and intramuscular EMG records (Fig. 3B). These data suggest that the afferent input and spinal excitability were insufficient to bring some motoneurons to threshold to fire in every contraction. A few other units were activated twice during one clonus cycle (Fig. 3C). However, most (98%) of the 166 motor units recorded from medial gastrocnemius during clonus fired once each cycle (Fig. 3A), indicating that the afferent activity from the previous contraction and the level of spinal excitation were adequate to recruit most of the units during every contraction but were insufficient to increase their firing rates. Similar single motor unit activity has been observed in the soleus muscle of uninjured humans during postural sway (34).

The time at which each motor unit was activated in different clonus cycles varied more than the changes in cycle duration (Fig. 5). Thus, only part of the scatter in the motor unit recruitment across cycles could be explained by the different conduction velocities of the various afferents (and efferents) excited during each cycle of clonus. It is likely that variations in the trajectory of the afterhyperpolarizations of the motoneurons and consequently the level of spinal neuron excitability (29) also contribute to the dispersal of activity in different motor units across cycles. Nevertheless, none of these peripheral or spinal factors were sufficient to markedly disrupt the recruitment order of pairs of motor units during clonus.

Another consideration is that the recruitment of motoneurons during clonus may be associated with the repeated activation of persistent inward currents, as is common in motoneurons of healthy rats, or those caudal to a chronic spinal cord injury (1, 2, 3, 26). Repeated stretches of muscle result in motor unit activity at low, regular rates (17, 23) that are close to the frequency of clonus. Slow, regular firing of motor units also occurs during and after tonic muscle spasms (45), and after voluntary contractions of muscles influenced by chronic human spinal cord injury and has been attributed to the activation of persistent inward currents in motoneurons (16, 53, 54). If these currents are also activated during clonus,
the afferent input generated by subsequent muscle contractions may then act to alter the regularity of this motor unit activity, varying the time at which units are recruited in different clonus cycles. Thus, motor unit activity at low regular rates due to repeated activation of persistent inward currents (and deactivation via the afterhyperpolarization of the motoneuron) may represent the central component of clonus proposed by others (4, 14, 48, 49) and underlie the rhythmic contractions seen after the first few cycles of clonus (Fig. 2).

Conclusion. We conclude that the orderly recruitment of motor units during clonus in muscles chronically paralyzed by injury to the human cervical spinal cord adds further support to the view that spinal mechanisms are critical for the relatively stereotyped sequence of motor unit recruitment seen during many voluntary contractions.
ACKNOWLEDGMENTS

The authors thank Dr. Galina Kozhina for her contributions. This research was funded by USPHS grant NS-30226, Faculty of Education, The University of Auckland, and The Miami Project to Cure Paralysis.
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Fig. 1A. Each subject sat in their wheelchair with the test foot resting on a wooden platform and secured with a Velcro strap. A transducer, which was housed in a wooden constraint beneath the platform, contacted the footrest at only one point. B. Unprocessed medial gastrocnemius surface EMG, torque and intramuscular EMG (top to bottom) during five clonus cycles. The onsets and offsets in surface EMG (dotted and dashed vertical lines) mark the EMG bursts and interbursts. Potentials from two units that fired in the same order each cycle are marked (• •) and overlaid to show accurate unit identification.
Fig. 2. Integrated medial gastrocnemius surface EMG during the bursts from one spasm (A), all six spasms involving clonus from the same experiment (B), and seven spasms involving clonus from another experiment on the same subject performed on a different day (C). D-F. Cycle and burst durations that correspond to the data shown in A-C. Each symbol (●) shows data from one clonus cycle.
Fig. 3. Unprocessed surface (top) and intramuscular EMG (bottom) from four cycles of clonus (A) to show that a unit (●) fired once per cycle. Overlays of the potentials (right) show accurate unit identification. Dotted and dashed lines mark the onset and offset of each burst of EMG. B. As in A, but one unit fires in every cycle (●; dotted lines show the unit potentials are in the intramuscular and surface EMG) while another unit fires in 7 of 9 cycles (*). C. Unprocessed surface EMG, intramuscular EMG and instantaneous frequency (top to bottom) for four clonus cycles to show one unit fires at clonus frequency in 3 cycles but is activated twice during the last cycle. A raster of the unit potentials is at the right.
Fig. 4. Instantaneous firing rate for 5 units during clonus (•; A-E) plotted with the corresponding cycle rate (solid line). The units in A and B were coactive in some cycles of clonus, as were the units in C-E but during a different spasm involving clonus.
Fig. 5. Coefficients of variation for mean unit firing rate versus those for cycle rate (n=166 units). Most of the data lie below the unity line indicating that cycle durations varied less than unit firing times.
Fig. 6A. Surface EMG, intramuscular EMG and torque (top to bottom) for 20 clonus cycles to show the fixed firing order among three units, each marked with a different symbol (+ • *). Overlays of potentials from each unit (right) show accurate unit identification. B. Percent recruitment reversals for 59 motor unit pairs versus mean recruitment time difference, expressed as milliseconds.
Fig. 7A. Unprocessed medial gastrocnemius surface EMG (top), averaged surface EMG (middle) and averaged intramuscular EMG (bottom) of a unit activated in the interburst at 82% cycle time. B. The same data as in A except that the averaged surface EMG and intramuscular EMG are for a different unit that fired at 20% cycle time. Dotted lines show where each unit fired relative to the clonus cycle.