

# Windup of Flexion Reflexes in Chronic Human Spinal Cord Injury: A Marker for Neuronal Plateau Potentials?

T. G. HORNBY,<sup>1,3</sup> W. Z. RYMER,<sup>1–3</sup> E. N. BENZ,<sup>3</sup> AND B. D. SCHMIT<sup>1,3,4</sup>

<sup>1</sup>Department of Physical Medicine and Rehabilitation and <sup>2</sup>Department of Physical and Biomedical Engineering, Northwestern University Medical School, Chicago, IL 60611; <sup>3</sup>Sensory Motor Performance Program, Rehabilitation Institute of Chicago, Chicago, Illinois 60611; and <sup>4</sup>Department of Biomedical Engineering, Marquette University, Milwaukee, Wisconsin 53201-1881

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**Hornby, T. G., W. Z. Rymer, E. N. Benz, and B. D. Schmit.** Windup of flexion reflexes in chronic human spinal cord injury: a marker for neuronal plateau potentials? *J Neurophysiol* 89: 416–426, 2003; 10.1152/jn.00979.2001. The physiological basis of flexion spasms in individuals after spinal cord injury (SCI) may involve alterations in the properties of spinal neurons in the flexion reflex pathways. We hypothesize that these changes would be manifested as progressive increases in reflex response with repetitive stimulus application (i.e., “windup”) of the flexion reflexes. We investigated the windup of flexion reflex responses in 12 individuals with complete chronic SCI. Flexion reflexes were triggered using trains of electrical stimulation of plantar skin at variable intensities and inter-stimulus intervals. For threshold and suprathreshold stimulation, windup of both peak ankle and hip flexion torques and of integrated tibialis anterior electromyographic activity was observed consistently in all patients at inter-stimulus intervals  $\leq 3$  s. For subthreshold stimuli, facilitation of reflexes occurred only at intervals  $\leq 1$  s. Similarly, the latency of flexion reflexes decreased significantly at intervals  $\leq 1$  s. Patients that were receiving anti-spasticity medications (e.g., baclofen) had surprisingly larger windup of reflex responses than those who did not take such medications, although this difference may be related to differences of spasm frequency between the groups of subjects. The results indicate that the increase in spinal neuronal excitability following a train of electrical stimuli lasts for  $\leq 3$  s, similar to previous studies of nociceptive processing. Such long-lasting increases in flexion reflex responses suggest that cellular mechanisms such as plateau potentials in spinal motoneurons, interneurons, or both, may partially mediate spinal cord hyperexcitability in the absence of descending modulatory input.

## INTRODUCTION

Uncontrolled flexion spasms are a common clinical manifestation of spasticity in individuals with chronic spinal cord injury (SCI), and these spasms frequently interfere with functional independence (Little et al. 1989). After recovery from spinal shock, many types of innocuous or noxious cutaneous or muscle stimuli to the lower limb can elicit a prolonged, coordinated pattern of hip flexion and ankle dorsiflexion, similar to flexion withdrawal reflexes (Schmit et al. 2000). Medical treatment of such involuntary responses typically involves pharmacological interventions targeted at the central or peripheral

neuromuscular apparatus, although the underlying mechanisms for such behaviors are unknown.

The predominant theory regarding the mechanism underlying these behaviors involves an increased excitability of spinal neurons after SCI. In particular, previous work in the decerebrate cat has suggested that hyperexcitability of interneuronal pools after acute complete or partial spinal section results in a transition from rigidity to excitable flexion reflex behaviors (Burke et al. 1972; Rymer et al. 1979). The exact nature of this hyperexcitability is unknown, but it is thought to be primarily a result of the release of interneuronal circuits from descending inhibitory influences (Engberg et al. 1968; Heckman 1994).

One possible mechanism for the observed increased excitability after SCI may be the manifestation of plateau potentials (PPs) in spinal cord neurons. In most vertebrate species, PPs are observed as voltage-gated sustained periods of depolarization or discharge that can amplify and prolong the effects of excitatory inputs (reviewed in Hultborn 1999; Kiehn and Eken 1998). PPs are also associated with “warm-up” or windup, in which repeated stimulation at intervals  $< 4$ – $6$  s progressively facilitates neuronal excitability (Bennett et al. 1998; Svirskis and Hounsgaard 1997). In motoneurons, manifestation of such nonlinear behavior typically requires descending or exogenous neuromodulatory input. Specifically, PP activity in motoneurons recorded from the decerebrate cat is abolished after spinalization and returns after administration of monoamines (Hounsgaard et al. 1988). The effects of monoaminergic agents differ between spinal neuron populations, however, exciting motoneuronal populations and inhibiting interneurons (Heckman 1994). Accordingly, interneurons can demonstrate cellular behaviors indicative of PPs, including long-lasting windup (Morisset and Nagy 1999; Russo and Hounsgaard 1994), after release of descending inhibition that occurs during acute spinalization.

The functional significance of PPs has remained questionable (Kiehn and Eken 1998) although their presence was inferred from motor unit recordings  $> 10$  years ago (Eken and Kiehn 1989) and more recently during normal motor behaviors (Eken 1998; Gorassini et al. 1999a), including in studies involving humans (Gorassini et al. 1998, 2000; Kiehn and Eken

Address for reprint requests: T. G. Hornby, Dept. of Physical Medicine and Rehabilitation, Northwestern University, 345 E. Superior St., Rm. 1406, Chicago, IL 60611 (E-mail: g-hornby@northwestern.edu).

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1997). Recent evidence from a minimally disruptive, chronically S<sub>2</sub>-spinalized adult rat indicates that PPs may be present in motoneurons without exogenous neuromodulation (Bennett et al. 2001) and contribute to spastic motor behaviors. One month after spinalization, hyper-reflexive behaviors of the tail appear, characterized by periods of hypertonicity, flexion and extensor spasms, and clonus, triggered either by muscle stretch or cutaneous inputs (Bennett et al. 1999a). Intracellular recordings from motoneurons innervating the tail of the adult rat after chronic spinalization reveal spontaneous PP behavior. Such behaviors were rarely present in the acutely spinalized state; rather, their manifestation usually required neuromodulatory input. The time frame for generation of spontaneous PPs is consistent with that of the spastic motor behaviors, indicating that PPs may underlie such abnormal motor activity. Similar findings were reported nearly 10 years earlier in the chronically spinalized adult cat (Eken et al. 1989) and were hypothesized to contribute to spasticity after neurological injury. In a recent study, motor-unit recordings during prolonged spasms in humans with SCI demonstrated behavior possibly indicative of underlying PPs (Gorassini et al. 1999b, 2000). Further, PPs have been implicated in prolonged motor output after low-intensity muscle stimulation in individuals with chronic SCI (Collins et al. 2001) with no mention of the contribution of the observed behavior to spasticity.

In this study, we investigated the possibility that PPs in spinal pathways contribute to the hyperexcitability of flexion withdrawal reflexes in human subjects with chronic SCI. As suggested previously (Eken et al. 1989), prolonged spastic motor behaviors (such as flexion withdrawal) that outlast synaptic excitation appear qualitatively similar to motoneuron behaviors with PPs in reduced preparations. We hypothesize that, if present, PPs contribute to the windup of such flexion reflexes at prolonged intervals, consistent with facilitation of plateaus in reduced preparations. Evidence of windup in chronic SCI would indicate the presence of a plateau-like phenomenon, and contribute to our understanding of the pathophysiology underlying spasticity.

A preliminary account of this work has been published previously in abstract form (Hornby et al. 2001)

## METHODS

### *Subjects*

Individuals were recruited into this study through the inpatient and outpatient clinics of the Rehabilitation Institute of Chicago. Inclusion criteria included a history of chronic SCI (>6 mo) at the 8th thoracic spinal cord level (i.e., T<sub>8</sub>) or higher and subject or physical-therapist report of spasms. Further, subjects were selected to participate only if sensation or volitional control was absent in their lower extremities (i.e., clinically complete lesions with American Spinal Injury Association Classification A). Exclusionary criteria included: multiple CNS lesion sites or secondary lesions of the cord; a history of peripheral nerve injury in both legs; the presence of significant complications such as skin breakdown or secondary infections; heterotrophic ossification in the lower extremities; significant osteoporosis; respiratory failure; presence of a cardiac pacemaker; or other concurrent illness limiting the capacity to conform with study requirements. Consent was obtained for each subject, and all procedures were conducted in accord with the Helsinki Declaration of 1975 and approved by the Institutional Review Board of Northwestern University.

Thirteen subjects with clinically complete, chronic SCI were en-

rolled in this study, 12 of whom (2 females, 10 males) met the inclusion criteria and were eligible to participate. The mean age of the subjects was 39.2 yr (range: 24–67), and mean duration since injury was 8.9 yr (range: 1.5–27.8). All subjects presented with lesions between the C<sub>5</sub> and T<sub>8</sub> spinal cord levels, and all reported spasms during the day. The Penn Spasm Frequency Scale (Penn 1988), a self-report rating, was used to estimate the number of spasms experienced by the subjects (scores of 0 = no spasms, 1 = spasms triggered only by stimulation, 2 = <1 spasm/h, 3 = >1 spasm/h, and 4 = >10 spasms/h). One patient reported a score of 1, four patients reported a score of 2, six patients reported a score of 3, and one patient reported a score of 4. At the time of the study, six subjects were medically prescribed anti-spastic agents to reduce the intensity and frequency of spasms. Four were prescribed a combination of baclofen (90–160 mg/day) and diazepam (3 subjects; 10–15 mg/day) or tizanidine (1 subject; 5 mg/day) while two subjects received diazepam only (15–20 mg/day). Patients were not instructed to alter their medication dosage or schedule prior to or during the experiment.

### *Experimental design*

The details of the experimental setup have been described previously (Schmit et al. 2000). Briefly, each participant was transferred to the adjustable-height chair of the testing apparatus (Biodex Rehabilitation/Testing System 2; Biodex Medical Systems, Shirley, NY). The foot of the tested extremity was placed in a footplate at seat height, attached to a 6-df load cell, and secured using a clamp placed on the dorsum of the foot and a heel strap. Angles of the hip (range: 75–110°), knee (85–135°), and ankle (105–125°) and segments lengths of the thigh, shank, and foot-to-load cell were determined to calculate joint torques using equations described previously (Schmit et al. 2000). All signals were low-pass filtered (200 Hz), and sampled at 500 Hz using data-acquisition cards (National Instruments, Austin TX) on a personal computer.

Surface electromyograms (EMGs) were recorded from the tibialis anterior (TA), medial gastrocnemius (MG)/soleus, rectus femoris (RF), and medial hamstrings (MH; semimembranosus/semitendinosus) in all 12 subjects. Active Delsys electrodes (model DE2.1, Delsys, Boston MA) were applied to lightly abraded, degreased skin over the respective muscle belly. The signals were amplified (10,000 times), filtered (20–450 Hz; Bagnoli 4, Delsys), and sampled at 1,000 Hz using the same computer system used for acquiring the torque data. Consistent with previous reports (Hiersemenzel et al. 2000), preliminary studies indicated minimal and/or inconsistent EMG activity of the contralateral TA and MG. Routine investigation of contralateral lower extremity EMG activity was therefore precluded.

Flexion reflexes were elicited by electrical stimulation through bipolar surface electrodes (Blue Sensor, Medicotest, Rugmarken, DK) placed 1 cm apart at the foot. In 10 subjects, stimulating electrodes were placed on the medial arch. In the remaining subjects, minimal responses were observed after medial arch stimulation, and electrodes were moved to the web space between the first and second digits. In contrast to previous results in neurologically intact humans (Sonnenborg et al. 2001), stimulation at the two different sites elicited qualitatively similar flexion withdrawal patterns, as measured by relative proportions of ankle, knee, and hip torques across the subjects.

Stimulation was triggered by a custom-made computer program and delivered through a constant current stimulator (Model DS-7A, Digitimer Stimulator, Hertfordshire, UK). The electrical stimulus train consisted of a 20-ms, 500-Hz pulse train composed of 10 monophasic pulses (each pulse of 1-ms duration). Stimulus-response curves were first generated by randomly varying the intensity of stimulation (0–20 mA at 5-mA intervals; 30–70 mA at 10-mA intervals) while recording EMGs and torques. Each stimulus train was repeated five times at each intensity, with a 20-s interval between stimuli. The minimum current at which EMG activity was observed in the TA after stimulation was defined as the reflex threshold current. An example of a

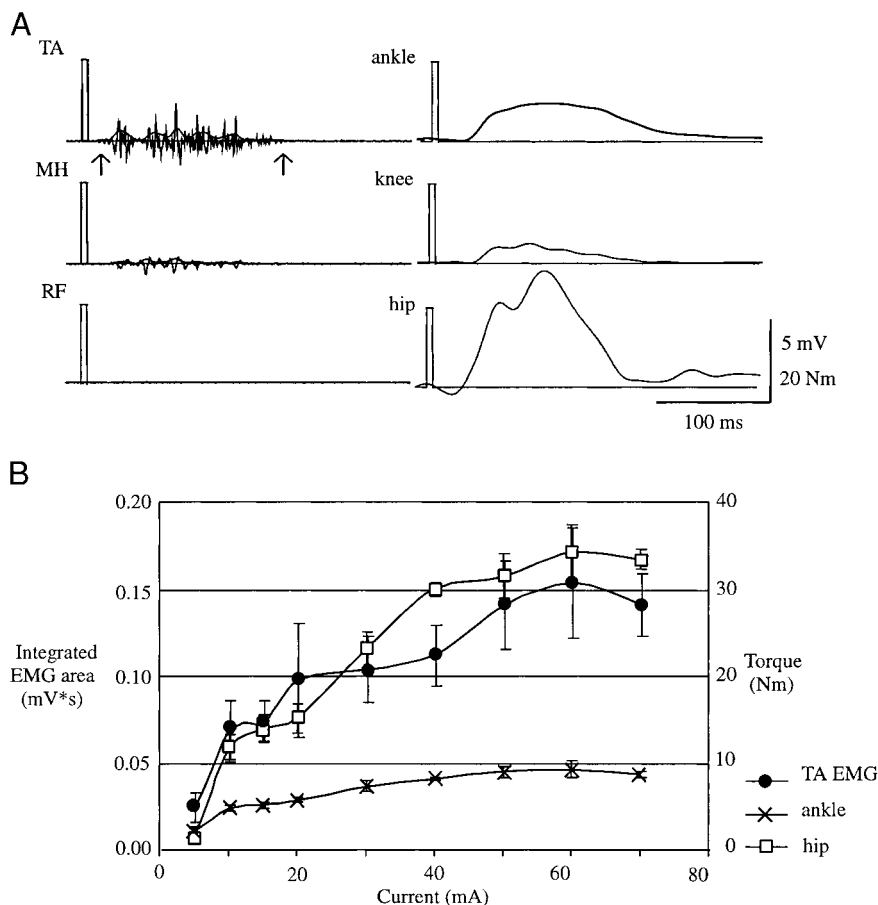


FIG. 1. A: electromyographic (EMG) activity from tibialis anterior (TA), rectus femoris (RF), and medial hamstring (MH) muscle groups and ankle dorsiflexion, knee and hip flexion torques after electrical stimulation of 50 mA at the medial arch of the foot. Robust responses were observed in recordings from the TA in all subjects with inconsistent responses from other muscles, as demonstrated here with recordings from the RF. Onset and offset times for the TA activity are indicated ( $\uparrow$ ). B: stimulus-response relation of current amplitude vs. mean integrated TA EMG and ankle and hip torques (error bars indicate SD). TA EMG activity was observed at threshold current intensities, with minimal responses from the ankle and hip. With stimulation at higher current intensities, TA EMG activity and ankle and hip torque all increased substantially, with responses typically reaching a plateau at  $>50$  mA (as noted with TA EMG and ankle torque).

typical flexion withdrawal response at 50-mA stimulation and the stimulus-response relation is provided in Fig. 1.

To examine the time-dependent variation in flexion reflexes, the intervals between stimuli were varied between 0.5 and 20 s. In all subjects, current intensities of 50 mA and reflex threshold current were repeated 5–10 times at inter-stimulus intervals of 0.5, 1, 2, 3, 5, and 20 s. In four subjects, additional stimuli were applied at random intervals between 0.5 and 60 s. A delay of  $>60$  s was provided between the trials of different inter-stimulus intervals.

#### Data collection and analysis

Elicitation of flexion reflex patterns was typically characterized by a rapid increase in EMG activity, followed by a slow decline to baseline (see Fig. 1). The onset and offset of activity were determined using MATLAB. The 60-Hz noise was removed from the EMG signals using a band-stop filter at 55–65 Hz (4th-order Butterworth filter applied backward and forward to remove phase delays). The signal was then rectified and smoothed (i.e., low-pass Butterworth filtered again applied forward and backward to remove phase delays). To detect the onset of EMG activity, a fourth-order 10-Hz filter was utilized to smooth the rectified signal. The time at which the rate of rise of the rectified signals reached a consistent threshold (first derivative of the TA EMG amplitude  $>2.5$  V/s) was detected across experimental trials and subjects. To detect the offset of EMG activity, a fourth-order 6-Hz filter was used to smooth the rectified EMG. The frequency cutoff to detect the onset of activity was higher than the cutoff for the offset because the EMG onset was sudden while the offset was often composed of small lingering bursts of activity. The time at which the rectified signal decreased  $<0.300$  mV above the mean baseline activity (determined from quiescent trials) was identified as the offset of EMG activity. Latency and duration of EMG

activity were detected from onset and offset signals, and the area of rectified, smoothed EMG activity during flexion reflexes was calculated.

Torque data were obtained for the hip, knee, and ankle after elicitation of flexion spasms. The signals were low-pass filtered at 25 Hz using a fourth-order Butterworth filter and plotted against time. Peak ankle, knee, and hip torque responses were identified for each subject.

Inconsistency of EMG activity detected in muscles other than TA and of knee torque responses prohibited their quantitative analysis. Integrated EMG activity, latency of TA EMG onset, and peak hip and ankle torques were determined for 10 subjects for all intervals at 50 mA and at reflex threshold current intensity. In one additional subject, only integrated TA EMG activity and latency were determined for trials at 50 mA, and this subject was not tested at reflex threshold current. In another subject, the reflex threshold current did not reliably generate a flexion withdrawal with TA activity.

The first five stimuli per trial were analyzed. Due to the variability in magnitude of responses between subjects, EMGs and torques were normalized to the initial (1st) response. Conversely, absolute values of the EMG latencies are reported. The focus of the analysis was on the temporal summation of these reflexes, which was typically maximal at the second and third responses. Each normalized second and third response were compared statistically to the first responses by use of a standard paired *t*-test, with significance noted at  $P < 0.05$  and  $P < 0.01$ .

#### RESULTS

In the present study, we investigated the withdrawal responses to repetitive cutaneous stimulation on the foot of

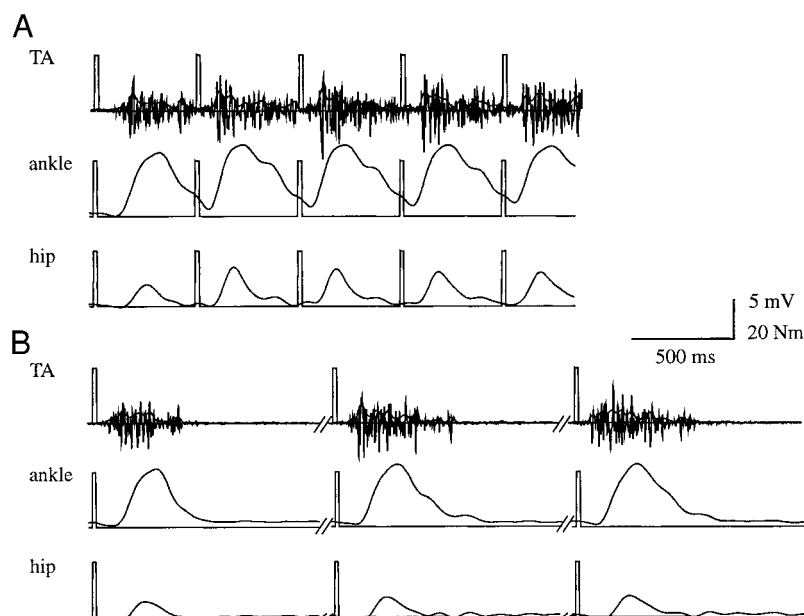


FIG. 2. Example of windup of TA EMG activity and ankle and hip flexion torques following repeated 50-mA stimulation at 0.5-s (A) and 3.0-s (B) intervals. Substantial increases in EMG and torques were observed across all subjects with repeated stimulation at shorter intervals ( $\leq 1$  s), with smaller increases at longer intervals.

subjects with complete chronic SCI. A stimulus-response relationship was first determined by stimulating at various current amplitudes (5–70 mA), while joint torques of the hip, knee, and ankle, and integrated EMGs of selected musculature were recorded. Single trains of stimuli were delivered at intervals between 0.5 s and 20 s, at subthreshold, threshold, and supratherreshold (50 mA) current intensities.

#### Stimulus response relationship

After a supratherreshold electrical stimulus, flexion reflexes were elicited, and these consisted of coordinated hip flexion and ankle dorsiflexion, with TA EMG activity present in all subjects tested. The typical EMG and joint torque responses for a flexion reflex triggered at a stimulus intensity of 50 mA, and the associated stimulus-response relation for one subject is presented in Fig. 1. Responses to stimulation were typically one of two types: a monophasic response of rapidly rising and slowly decaying EMG and torque or a bursting pattern of activity. Both types of flexion withdrawal responses in individuals after SCI have been reported previously (Remy-Neris et al. 1999; Shahani and Young 1971).

Figure 1A shows a typical example of the variability of motor responses after elicitation of flexion withdrawal in a single subject. In subjects demonstrating flexion reflexes, the large amplitude of TA EMG activity is consistent across all responses. For the data in Fig. 1A, the MH activity was substantial while RF EMG activity was minimal. Further, activity from the MG was small or absent in this and all other patients (data not shown). Of the torques generated after stimulation, hip flexion and ankle dorsiflexion were observed consistently across all subjects. Knee torque was typically inconsistent and smaller in comparison.

Figure 1B demonstrates the stimulus-response relation for an individual subject in which TA EMG activity and hip and ankle torques, averaged across five trials at 20-s intervals, increased with increasing current intensity. Threshold currents averaged  $16.67 \pm 5.77$  (SD) mA (range: 5–30). EMG activity recorded from the TA muscle and a corresponding ankle dorsiflexion

torque were the earliest signs of flexion withdrawal at low stimulus amplitudes. Hip torques were predominant at higher stimulus intensities, while ankle dorsiflexion torque generally reached a plateau at intensities  $>50$  mA.

#### Windup for supratherreshold stimuli

To investigate the history-dependent nature of the flexion reflexes, stimuli were applied at randomly selected intervals of 0.5–20 s at 50 mA for 12 subjects (mean: 3.33 times threshold). Figure 2 demonstrates the EMG and torque responses of one subject to repeated 50-mA stimuli applied at intervals of 0.5 and 3 s. At 0.5-s intervals, the amplitude and duration of TA EMG activity increased substantially with repeated stimuli. Joint torques at the ankle and hip underwent similar increases in magnitude at short inter-stimulus intervals, increasing in these examples to  $\sim 120$ – $140\%$  of their first stimulus values. For stimuli delivered every 3 s, changes in EMG activity and joint torques were not as dramatic as observed following stimuli delivered every 0.5 s. Specifically, there were notable increases in TA EMG and ankle and hip torques with repeated flexion reflexes although not as pronounced as at the shorter intervals.

The effects of repeated activation of flexion reflexes across all subjects at varying stimulus intervals are shown in Fig. 3 and Table 1. Figure 3 shows averaged normalized TA EMG activity and ankle and hip flexion torques across five sequential stimuli at 0.5- to 20-s intervals. Significant increases in both normalized EMG and torques are evident at 0.5- and 1-s intervals, particularly at the second and third responses, as noted in Table 1. With repeated large flexion reflexes, torque at both the ankle and hip increased  $\leq 160$ – $180\%$  of that normally achieved with single stimuli. After such increases, joint torques declined substantially with repeated stimuli. The decline in ankle joint torque was striking considering the maintenance of elevated excitability of the TA at short intervals, indicating possible excitation-contraction failure or metabolic fatigue (Gandevia et al. 1995).

At longer stimulus intervals of 2–3 s, increases in TA EMG



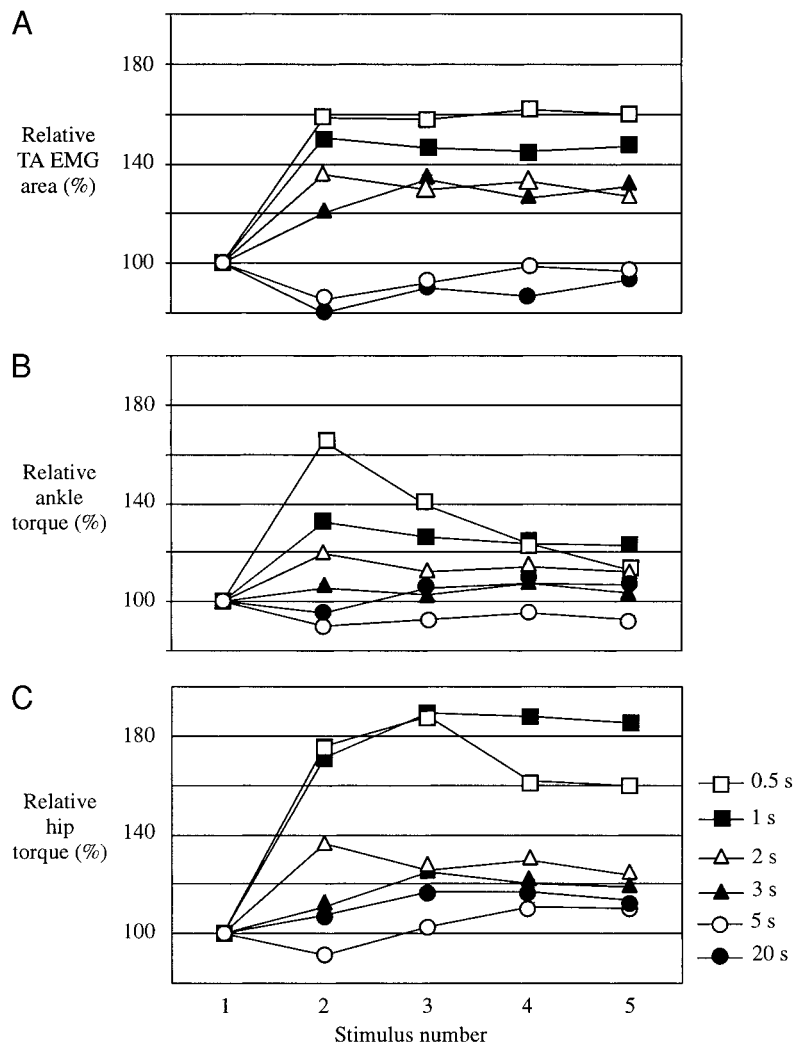


FIG. 3. Relative changes of mean TA EMG activity (A), and ankle (B) and hip (C) torques with repeated 50-mA stimulation at various intervals. Data combined from all 12 subjects for TA EMG activity and 10 subjects for torque responses. Significant differences were observed at intervals  $\leq 3$  s for TA EMG activity and  $\leq 2$  s for joint torques.

and hip joint torques were modest in comparison to results at  $\leq 1$  s. These responses were still consistent and often reached statistical significance (see Table 1). Specifically, at 2-s intervals, both TA EMG activity and hip torque increased rapidly after repeated stimulation with significant differences noted at the second stimulus. In contrast, stimuli applied at 3-s intervals

increased at a slower rate, with significant changes in TA activity noted at the third stimulus. Surprisingly, increases in ankle flexion torque reached 120% of the first stimulus level at 2 s but did not change substantially at 3-s intervals. At 5- and 20-s intervals, there was no significant change observed in TA EMG or hip and ankle torque with repeated stimulation.

TABLE 1. Wind-up of flexor reflexes with repeated suprathreshold and threshold stimuli

	Interstimulus Intervals, s					
	0.5	1.0	2.0	3.0	5.0	20.0
50 mA						
TA EMG	151 $\pm$ 47**	158 $\pm$ 33**	136 $\pm$ 31**	120 $\pm$ 31†	85 $\pm$ 30	80 $\pm$ 47
Ankle torque	165 $\pm$ 84**	132 $\pm$ 20**	120 $\pm$ 20†	105 $\pm$ 16	90 $\pm$ 15	96 $\pm$ 18
Hip torque	175 $\pm$ 68†	171 $\pm$ 88†	136 $\pm$ 52*	111 $\pm$ 36	85 $\pm$ 24	96 $\pm$ 38
Threshold						
TA EMG	265 $\pm$ 201**	175 $\pm$ 56**	161 $\pm$ 90**	142 $\pm$ 58*	105 $\pm$ 40	
Ankle torque	177 $\pm$ 64*	147 $\pm$ 35**	144 $\pm$ 39**	110 $\pm$ 41	97 $\pm$ 92	
Hip torque	288 $\pm$ 300	255 $\pm$ 256	142 $\pm$ 82	85 $\pm$ 56	94 $\pm$ 51	

Relative mean  $\pm$  SD changes (normalized to the 1st response) in tibialis anterior (TA) electromyographic (EMG) activity and ankle and hip torque at the 2nd stimulus at various intervals are shown. Significant differences are denoted at  $P < 0.05$  (\*) and  $P < 0.01$  (\*\*). Significant changes on the 3rd but not 2nd stimulus are indicated at  $P < 0.05$  (†). Specifically, integrated TA EMG increased to  $137 \pm 37\%$  (mean  $\pm$  SD) of control values at 3-s intervals, ankle torque increased to  $112 \pm 18\%$  at 2-s intervals, and hip torque increased to  $188 \pm 108\%$  and  $189 \pm 112\%$  at 1.0- and 0.5-s intervals, respectively. Despite the large increases in hip torque noted during repeated threshold stimulation at  $< 2$ -s intervals, variability between subjects precluded their statistical significance ( $P = 0.07$  at 0.5- and 1.0-s intervals).

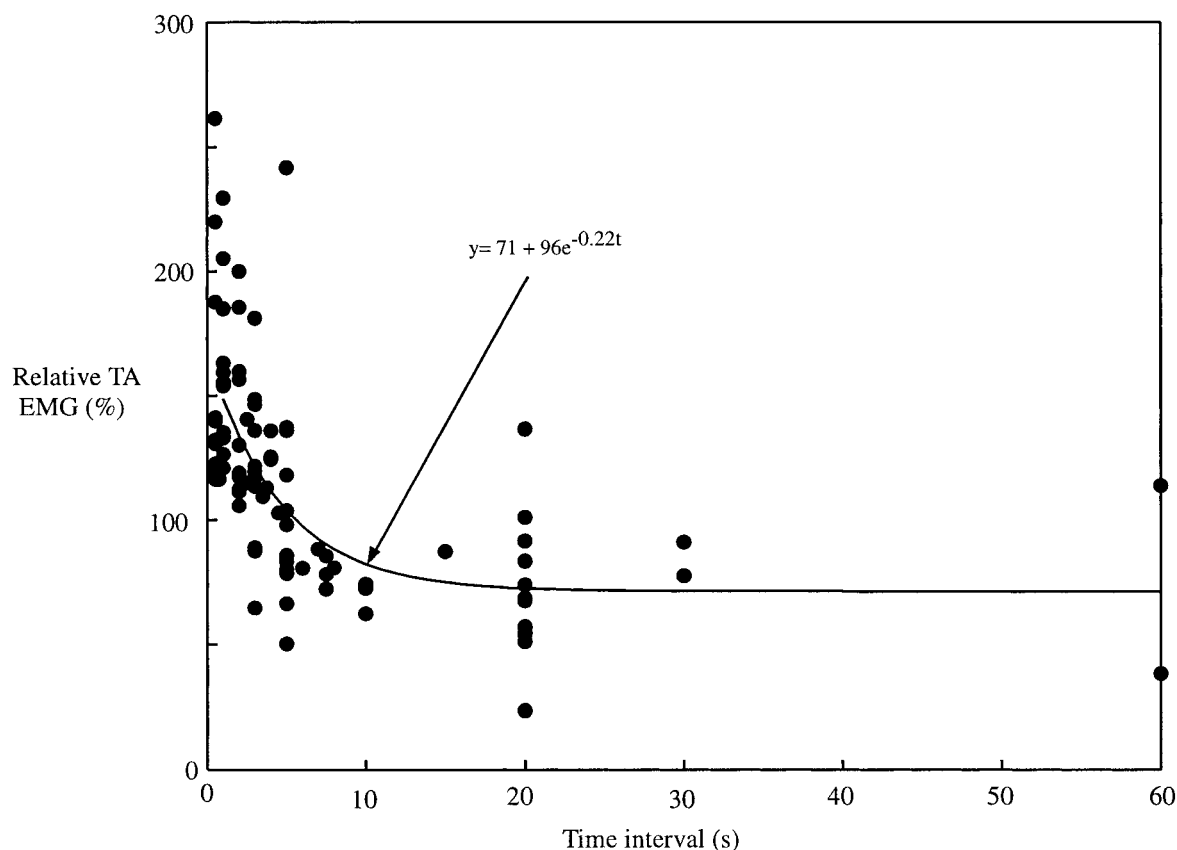


FIG. 4. Time course of windup of TA EMG activity with repeated stimulation. Integrated TA EMG area of the 2nd stimulus (normalized to the first stimulus) was plotted against the interstimulus interval duration across all subjects (0.5–20 s; as in Fig. 3) and from 4 subjects stimulated at additional time intervals (0.75–60 s). Data was fit with an exponential regression line revealing a time constant of  $\sim 4.7$  s.

The rapid rise and subsequent decline of EMG and torques demonstrated in Fig. 3 indicate that both adaptive and facilitative processes contributed to changes in reflex responses with repeated stimulation. To assess the time course of altered spinal excitability, the relative changes in TA EMG activity at the second stimulus for all subjects at all intervals (0.5–60 s) were combined, including the data from four subjects in which random intervals were applied. Figure 4 shows the exponential decay of the excitability of flexor reflexes with a time constant of 4.7 s. This time course of decay is similar to that of PP-generated windup of spinal cord neurons in reduced preparations (Bennett et al. 1998; Svirskis and Hounsgaard 1997). Notably, the TA EMG activity did not recover to 100% of its initial value but rather declines even with long-duration intervals. Such a decline of reflex excitability was observed in every experimental session as has been reported previously (Dimitrijevic and Nathan 1968).

#### Windup with threshold and subthreshold stimuli

Windup of flexion reflexes was also noted for stimuli applied just at or even below the reflex threshold current. Figure 5A shows the TA EMG and ankle dorsiflexion and hip flexion torques of one subject in response to stimuli at threshold intensity (10 mA), delivered at 0.5-s intervals. Substantial increases in joint torques and corresponding TA EMG activity were evident at short intervals and consistent across the population of subjects. Combined data of the normalized increases

in TA EMG activity with repeated threshold stimulation are presented in Fig. 5B. Quantitative comparisons of TA EMG and ankle and hip joint torques are provided in Table 1. In contrast to repeated 50-mA stimulation, threshold stimuli elicited relatively greater responses as normalized to the first responses, which was likely due to the smaller initial responses. Significant increases were, however, present at intervals  $\leq 3$  s.

Ankle and hip torques measured during repeated threshold stimulation were more variable than TA EMG activity. In particular, ankle torques increased significantly for intervals  $\leq 2$  s but not at 3-s intervals. Changes in hip torques, however, were not statistically significant at any of the stimulus intervals, likely due to the large variability of responses recorded from different subjects (see Table 1). For example, in one subject hip torque increased nearly 10 times greater than the response generated at threshold, whereas in another, very little hip torque was observed.

In previous studies using reduced preparations, repeated or prolonged subthreshold stimuli elicited delayed depolarization or discharge of dorsal interneurons and  $\alpha$  motoneurons dependent on underlying PPs (Bennett et al. 1998; Russo and Hounsgaard 1994; Svirskis and Hounsgaard 1997). To investigate whether similar behaviors could be demonstrated following SCI in humans, we delivered repeated subthreshold electrical stimuli at multiple intervals to seven subjects. Stimuli delivered at 5–10 mA below threshold [mean  $7.1 \pm 2.7$  (SD) mA;

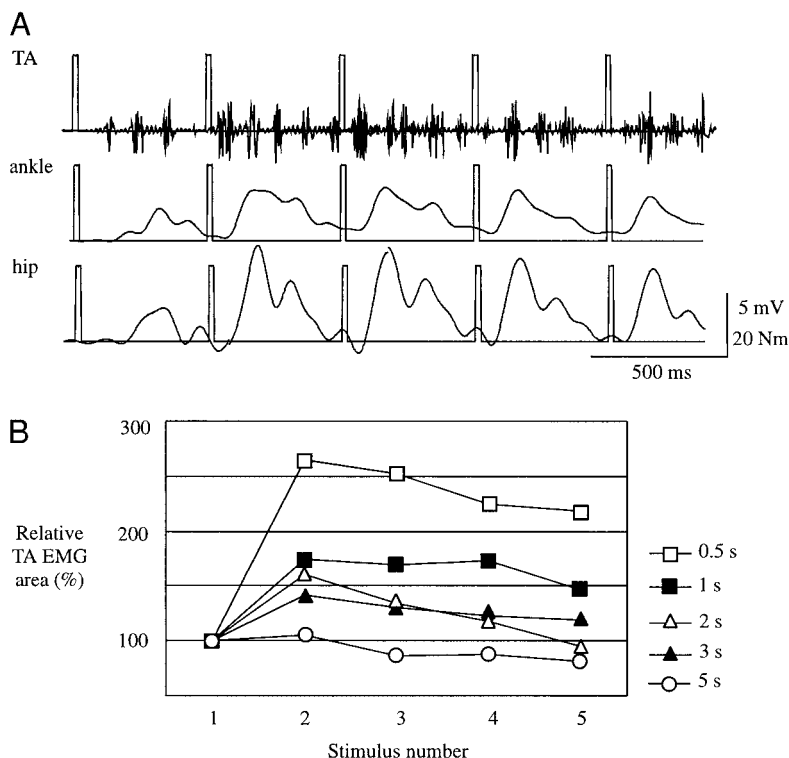


FIG. 5. A: example of windup of TA EMG activity and ankle and hip flexion torques after repetitive stimulation at 0.5-s intervals using threshold current. B: relative mean changes in TA EMG activity with repeated stimulation at various intervals across 10 subjects. As compared with the initial response, larger relative increases in torques and EMG activity were generated, with significant differences observed at intervals  $\leq 3$  s.

range: 5–10 mA] generated no responses at the first interval but elicited responses with subsequent stimuli.

To illustrate this phenomenon, the reflex responses of a subject after application of repeated subthreshold current pulses at 0.5- and 1.0-s intervals are shown in Fig. 6. At shorter intervals, repeated stimuli elicited substantial EMG activity in the TA, with large ankle and hip torques approaching 80% of those values generated following single 50-mA stimuli. By the third stimulus in all seven subjects, subthreshold stimuli at 0.5-s intervals generated an average of  $38 \pm 27\%$  (mean  $\pm$  SD) of the EMG activity noted in TA for responses generated at a single 50-mA stimulus. As shown in Fig. 6, the windup is reduced at 1-s intervals, with mean TA EMG activity reaching

only  $20 \pm 17\%$  of that elicited with single 50-mA stimuli. As expected, torque responses at both the hip and ankle increased as well ( $\sim 30\%$  of responses at single 50-mA stimuli). Responses to subthreshold stimuli were typically absent at intervals  $> 1$  s. In one case, however, we observed facilitation at  $\leq 3$ -s intervals as has been presented anecdotally by Dimitrijevic and Nathan (1968). We found this to be an exception to the population behavior, however.

#### Reduction in response latency with repeated stimulation

Previous studies of the windup phenomenon in models of nociception have demonstrated a decrease in time to onset of electrophysiological responses with repeated stimulation in reduced preparations (Herrero et al. 2000). To assess whether this phenomenon occurred during repetitive triggering of flexion reflexes in chronic SCI, we assessed the latency of TA responses during repeated stimulation at both 50-mA and reflex threshold current. We found that there was a consistent reduction in latency with repeated stimulus application. An example of a decrease in latency is provided in Fig. 7A, with a more rapid onset of EMG activity with repeated stimuli applied at 0.5-s intervals.

Quantification of the reduction in latency on reflex onset with variable inter-stimulus intervals revealed that the most pronounced decrease occurred at intervals  $\leq 1$  s. Figure 7B demonstrates this variation, in which the mean onset of flexion reflexes decreased from  $92 \pm 65$  to  $47 \pm 11$  (SD) ms on the second stimulation using 0.5-s intervals and  $95 \pm 44$  to  $53 \pm 16$  ms with 1-s intervals (both at  $P < 0.01$ ). The observed decrease in latency observed at 2.0-s intervals during 50-mA stimuli was modest but not statistically significant ( $P = 0.05$ ). Similar behaviors were observed at threshold current intensities, with significant decreases in latency only at 0.5 s (119 to 48 ms) and 1-s intervals (104 to 61 ms; both at  $P < 0.01$ ). Such

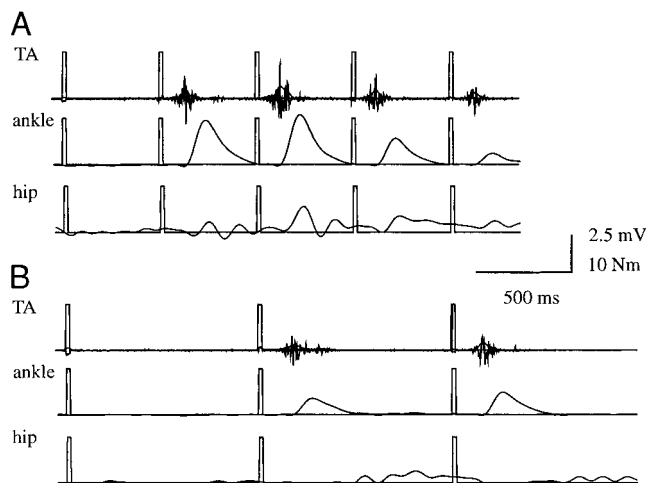


FIG. 6. Example of windup of TA EMG activity and ankle and hip flexion torques after repetitive subthreshold stimulation at 0.5-s (A) and 1.0-s (B) intervals. Greater windup was observed at 0.5 s with similar changes noted in all 7 patients tested. Windup was typically absent with repeated stimuli delivered at intervals  $> 1$  s.

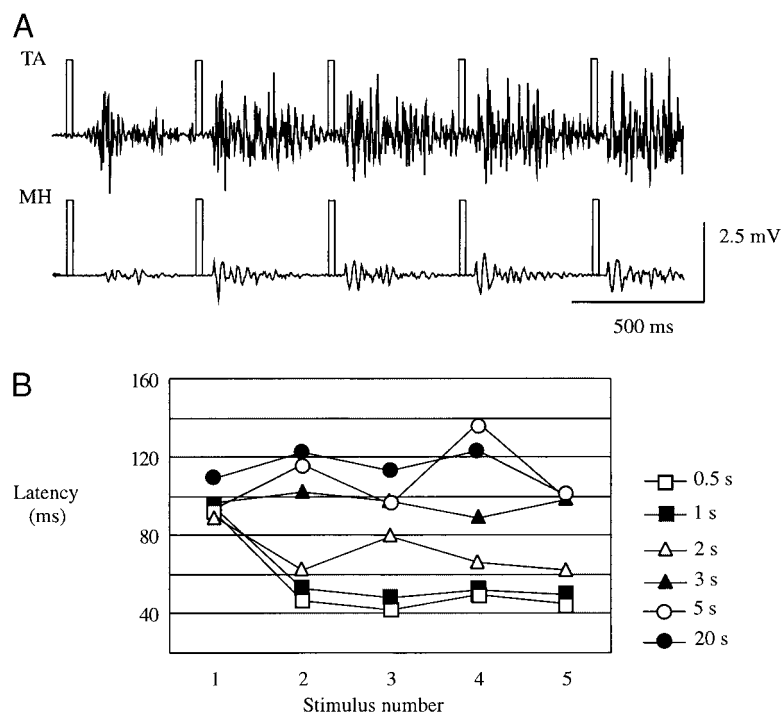


FIG. 7. A: example of the rapid decrease in latency of EMG activity onset in TA and MH muscle groups after repeated 50-mA stimulation at 0.5-s intervals. B: mean changes in latency of TA EMG onset after repeated 50-mA stimulation at various intervals. Significance was noted at intervals  $\leq 1$  s with similar changes noted with repeated threshold stimulation.

large reductions in response onset may indicate a reduction in duration of spinal processing of afferent information and/or a change in the pathway of transmission for these afferent responses from slower (type C) to faster (type A $\beta$ ) conducting nerve fibers (Schouenborg and Sjolund 1983).

#### Effects of anti-spasticity medications and generation of windup

Baclofen, a GABA<sub>B</sub> agonist commonly prescribed to patients with uncontrolled spasms after SCI, has been shown to decrease PP behavior in ventral horn neurons the adult turtle (Svirskis and Hounsgaard 1998). The effects of other anti-spastic agents, in particular diazepam and tizanidine, on PP activity is unknown, although both have been shown to reduce spinal neuronal excitability through GABA<sub>A</sub> and adrenergic  $\alpha_2$  receptors, respectively.

To investigate the extent to which these various anti-spastic medications reduce windup, flexion withdrawal responses of subjects prescribed anti-spastic medications were grouped together and compared with the responses of subjects not taking such medications. Surprisingly, windup of the second response in subjects on medications was 30% greater on average than that of the subjects not prescribed anti-spastic agents. Similarly, with threshold stimulation, windup was nearly 50% less in subjects not receiving medications at 0.5-s intervals and 35% less at 1-s intervals. Differences between the groups were not statistically significant ( $P \geq 0.10$  at all intervals), likely due to the small size of each group ( $n = 6$ ). A possible explanation for this result may have been the different levels of spasticity experienced by the two patients groups. Indeed, the mean Penn score for patients receiving anti-spastic medication was slightly higher (3.0 vs. 2.17; median: 3.0 vs. 2.0), although their reflex threshold currents were slightly higher on average (14.1 vs. 10.1 mA). There was no apparent relation between the reflex threshold current, medication usage, Penn Spasm Frequency

scores, and the level of injury. The limited number of patients in each category precluded further analysis of any difference; however, the spasms experienced by the subjects may be linked to the extent of windup of flexion reflexes.

#### DISCUSSION

In this study, we have provided quantitative evidence of windup of flexion reflexes in individuals with chronic SCI with repeated stimulation, as reported anecdotally by others (Dimitrijevic and Nathan 1968, 1970; Shahani and Young 1971). With repeated threshold and suprathreshold stimuli, significant increases in TA EMG activity and ankle and hip torques were demonstrated at intervals  $\leq 3$  s. At lower stimulation intensities, higher frequencies were required to facilitate reflexes, indicating that windup was dependent on both stimulus intensity and interval. The latency of EMG onset was also reduced significantly with stimuli applied at  $\leq 1$  s, suggesting a possible change in afferent pathways mediating flexion reflexes. The extent of windup was unexpectedly greater in subjects prescribed anti-spasticity medications, although this may be attributed partly to differences in the severity of spasticity in the two groups.

Prolonged flexion reflexes in humans with SCI have been attributed to increased hyperexcitability of spinal cord circuitry, although the cellular mechanisms underlying such behaviors are unknown. Long-lasting reflex activity in experimental models of chronic SCI has previously been attributed to underlying PP behavior (Bennett et al. 1999a,b; Eken et al. 1989), and the qualitative similarities between the phenomena are remarkable. Here, we provide quantitative evidence that flexion reflexes demonstrate another characteristic of PP behavior, specifically, long-lasting facilitation of excitability (i.e., windup). Such cellular properties may be responsible for hyperexcitable spinal circuitry after injury and are similar to those mechanisms responsible for windup of nociceptive informa-



tion. Specifically, both behaviors share properties consistent with underlying PP behavior. Knowledge of the cellular mechanisms underlying spasms may provide a basis for future physical and pharmacological interventions for individuals with SCI.

### *Mechanisms underlying windup of flexion reflexes*

Facilitation of flexion withdrawal reflexes at intervals  $\leq 3$  s is consistent with previous studies on windup in response to nociceptive stimuli in both intact and reduced preparations. Early studies determined that repeated C-fiber input at  $>0.3$  Hz is necessary to generate windup of dorsal lateral ascending tracts (Mendell 1966) and flexion reflexes (Price 1972) in acute spinal cats. Numerous researchers have since attempted to uncover the physiological mechanisms underlying frequency-dependent facilitation of nociceptive information (reviewed in Baranauskas and Nistri 1998; Herrero et al. 2000), with both presynaptic and postsynaptic mechanisms suggested to play a role. While presynaptic facilitation could contribute to the phenomena observed in this study, the marked reduction of windup in reduced preparations with manipulation of postsynaptic mechanisms (as described in the following text) has led us to consider the latter as a more likely candidate.

Possible postsynaptic mechanisms underlying windup include the contribution of *N*-methyl *D*-aspartate (NMDA) receptors, summation of slow excitatory potentials mediated by neuropeptides (e.g., substance P), and/or intrinsic  $\text{Ca}^{2+}$  conductances responsible for PPs (Baranauskas and Nistri 1998). While application of specific antagonists to substance P and NMDA reduces windup (Baranauskas et al. 1995; Barbieri and Nistri 2001; Davies and Lodge 1987; Dickenson and Sullivan 1987), NMDA currents decay after 100–300 ms in spinal neurons (Dale and Grillner 1986; Dale and Roberts 1985; see comment by Currie and Stein 1988). Further, substance P has been shown to enhance PPs (Russo and Hounsgaard 1997), indicating that these modulatory pathways may not be mutually exclusive.

The present results revealed a time course of facilitation that is most similar to results from reduced preparations in which PPs are directly observed or tested for. For example, studies in the *in vitro* rat spinal cord demonstrated windup in  $\sim 30\%$  of deep (lamina V) dorsal horn neurons using dorsal root and intracellular stimulation at 0.4–1.0 Hz (1.0–2.5 s intervals) (Morisset and Nagy 1998, 1999). Windup at these intervals was similar to that observed in turtle (Svirskis and Hounsgaard 1997) and cat motoneurons (Bennett et al. 1998) and did not change following blockade of NMDA receptors (Morisset and Nagy 2000). These cells were shown to possess the L-type  $\text{Ca}^{2+}$  current thought to be primarily responsible for PP formation (Morisset and Nagy 1999). Blockade of this current by nifedipine decreased PP activity and windup in both rat (Morisset and Nagy 2000) and turtle dorsal horn neurons (Russo and Hounsgaard 1994, 1996). Further, in a preliminary study of flexion reflexes in intact, 3-wk-old rats (Sibon et al. 1999), application of nifedipine reduced windup by 90% without alteration of the first response. Such studies indicate a predominant role of nifedipine-sensitive PPs in the facilitation of responses to repeated stimuli delivered within 3 s, as described in this study.

### *Neural substrates underlying windup of hyperexcitable reflexes after SCI*

Hyperexcitable interneurons have long been postulated to contribute to exaggerated flexion reflexes after acute partial or complete SCI in the decerebrate cat preparation (Engberg et al. 1968). For example, brief activation of mechanosensitive muscle free nerve endings produces prolonged activity in interneurons from lamina V–VII that corresponds with prolonged flexor activity and extensor inhibition (Cleland and Rymer 1990, 1993; Cleland et al. 1990). Such prolonged interneuronal discharge after a brief stimulus (cf., Chen et al. 2001) is strikingly similar to PP behavior in spinal neurons (e.g., Lee and Heckman 1998). While these interneurons respond to both muscle stretch and contraction, they are also excited by a wide variety of noxious and innocuous modalities (Cleland and Rymer 1993). Similarly, deep dorsal horn neurons that demonstrate PPs spontaneously in the aforementioned *in vitro* rat spinal cord studies are multi-receptive, or wide dynamic range, cells (Morisset and Nagy 1999). These particular cells demonstrate the greatest windup at frequencies  $>0.3$  Hz, similar to the time course of windup of flexion reflexes (Schouenborg and Sjolund 1983). It is therefore likely that the multi-receptive interneurons responsible for exaggerated flexion withdrawal (in the *in vivo* cat after spinal hemisection) are the same class of cells with identified PPs responsible for the windup of flexor reflexes (in the *in vitro* rat spinal cord).

In this study, the evidence that multi-receptive, PP-generating interneurons contribute to facilitation of prolonged flexion reflexes is twofold. First, low-threshold (i.e.,  $\sim 5$  mA), non-noxious, single or repeated stimuli were sufficient to elicit flexion reflexes in 5/12 patients, consistent with a reduction in threshold of reflexes in subjects with SCI (Shahani and Young 1971). Second, as discussed previously (Dimitrijevic and Nathan 1970), the decrease in latency of flexion reflex onset with repeated stimuli at  $\leq 1$ -s intervals suggests that large sensory fibers mediate such responses. In reduced preparations, short-latency reflex responses, likely mediated by  $A\beta$  fibers, have been observed with stimulation at intensities sufficient to activate C fibers (Schouenborg and Sjolund 1983). The multi-receptive cells likely contributing to hyperexcitable flexion withdrawal may initially respond to high-threshold stimuli mediated by  $A\delta$  or C fibers and subsequently relay information through  $A\beta$  fibers with repeated stimuli. One drawback to this hypothesis is that low-threshold stimuli transmitted by  $A\beta$  fibers generated long latency flexion withdrawal reflexes on the first response in some subjects. It is likely that long-lasting spinal processing of afferent information contributes to the initial long latency of responses but repeated stimuli reduce the duration of this processing.

With our stimulation paradigm, we cannot exclude the role of PPs in motoneurons in the facilitation of flexor reflexes. As described previously, spastic motor behaviors of the tail were observed at 1 mo after spinal transection at the  $S_2$  level in the adult rat (Bennett et al. 1999a) and consistent with the onset of spontaneous PP generation in tail motoneurons recorded *in vitro* (Bennett et al. 1999b, 2001). Two recent studies in humans with SCI have attributed involuntary motor behaviors to motoneuronal PPs (Collins et al. 2001; Gorassini et al. 1999b, 2000). In these reports and the present work, the evidence for the presence and locus of PPs was necessarily indi-

rect and the data can similarly be attributed to interneuronal PPs. At this point, detailed electrophysiological investigation of both types of spinal neurons in reduced preparations after acute and chronic SCI is necessary to understand their relative contribution toward spastic motor behaviors.

### *Relation to spasticity and pharmacological interventions*

The observation that windup of flexion reflexes was greater in individuals prescribed medications to manage their spasticity was surprising, considering the depressive effects of baclofen (Svirskis and Hounsgaard 1998) and possibly tizanidine (Heckman 1994) on PPs and on neuronal excitability in general (diazepam). This difference was at least partly accounted for by the patients' reports of spasm frequency (Penn 1988). This subjective measure considers only frequency and not intensity of spasms, however, and is poorly correlated with other clinical measures of spasticity (Priebe et al. 1996). In a previous study examining the contribution of PPs underlying spasms in human SCI (Gorassini et al. 2000), there was no indication of an association between PP behavior and the magnitude of spasms. While our results on this relationship are anecdotal and speculative, clinical assessment of spasticity specific to SCI, combined with electrophysiological and biomechanical quantification of flexion reflex facilitation, could establish whether this association is truly valid.

In conclusion, we have described the temporal facilitation of flexion withdrawal reflexes at both threshold and suprathreshold trains of electrical stimuli. Windup of reflexes was significant at stimulation intervals  $\leq 3$  s, indicating a long-lasting storage of excitability similar to PPs. Furthermore, temporal summation was dependent on both the amplitude and interval duration of the repeated stimuli. While multiple mechanisms likely play a role, we argue that the observed behaviors were mediated in part by PPs in the spinal interneuronal, and possibly motoneuronal circuitry, which are manifested after SCI.

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