Synchronous and asynchronous electrically evoked motor activities during wind-up stimulation are differentially modulated following an acute spinal transection

Alain Frigon,¹ Marie-France Hurteau,¹ Michael D. Johnson,² C. J. Heckman,²,³ Alessandro Telonio,¹ and Yann Thibaudier¹

¹Department of Physiology and Biophysics, Faculty of Medicine and Health Sciences, Centre de recherche Clinique Étienne-Le Bel, Université de Sherbrooke, Sherbrooke, Quebec, Canada; ²Department of Physiology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois; and ³Department of Physical Medicine and Rehabilitation, Physical Therapy and Human Movement Sciences, Northwestern University, Feinberg School of Medicine, Chicago, Illinois

Submitted 7 August 2012; accepted in final form 17 September 2012

Frigon A, Hurteau MF, Johnson MD, Heckman CJ, Telonio A, Thibaudier Y. Synchronous and asynchronous electrically evoked motor activities during wind-up stimulation are differentially modulated following an acute spinal transection. J Neurophysiol 108: 3322–3332, 2012. First published September 19, 2012; doi:10.1152/jn.00683.2012.—In this study, we used a novel technique to study reflex wind-up when the spinal cord is intact and following an acute spinal transection. Specifically, we evaluated reflex responses evoked by a series of 10 electrical pulses to the tibial or superficial peroneal nerves in 9 decerebrate adult cats, before and after an acute spinal transection. Electromyograms were recorded in four hindlimb muscles (lateral gastrocnemius, tibialis anterior, semitendinosus, and sartorius) to evaluate reflex amplitude, duration, and the temporal summation of reflex responses, so-called wind-up. We identified two distinct reflex responses evoked by electrical stimulation of the tibial or superficial peroneal nerves on the basis of their pattern of change following acute spinal transection, a short-latency (~10 ms) compound action potential (CAP) that was followed by a burst of sustained activity (SA). Wind-up of CAP and SA amplitudes was clearly present when the spinal cord was intact but was drastically reduced after acute spinalization in some muscles. Moreover, CAP and SA reflex responses were differentially modified by the acute spinalization. When the effects of acute spinal transection were significant, CAP responses were increased after acute spinalization, whereas SA responses were reduced, suggesting that the two signals are regulated by different neuronal mechanisms. The present results provide the first assessment of reflex wind-up before and after an acute spinal transection in the same animals and indicate that different reflex components must be considered separately when evaluating changes in neuronal excitability following SCI.

spinal cord injury; reflex; wind-up

THE COMPLETE LOSS OF DESCENDING pathways from supraspinal structures, such as occurs following spinal transection (i.e., spinalization), produces widespread functional changes in simple (e.g., reflexes) and complex (e.g., pattern generators) neuronal circuits within the spinal cord (reviewed in Edgerton et al. 2004; Frigon and Rossignol 2006; Hultborn 2003; Rossignol et al. 2008; Rossignol and Frigon 2011). Such changes are due to alterations in various excitatory and inhibitory connections and also, in part, to modifications in the intrinsic properties of neurons that compose these circuits. For instance, the ability of neurons to generate sustained firing is lost or considerably depressed in the early stages after spinal transection (Bennett et al. 2001b; Hounsgaard et al. 1988). Sustained neuronal firing is mediated by persistent inward currents (PICs) via voltage-dependent Na⁺ and Ca²⁺ channels that inactivate slowly (reviewed in Alaburda et al. 2002; Brownstone 2006; Heckman et al. 2003, 2008; Hultborn et al. 2004; Perrier et al. 2002). PICs are strongly facilitated by neuromodulators, such as serotonin and norepinephrine (Conway et al. 1988; Crone et al. 1988; Harvey et al. 2006; Hounsgaard et al. 1988; Lee and Heckman 1999; Li et al. 2007), which are released by pathways originating in the brain stem (Jordan et al. 2008; Rekling et al. 2000). After complete spinal cord injury (SCI), the release of neuromodulators within the spinal cord is greatly reduced, and although neuronal PICs are initially lost, they recover with time (Bennett et al. 2001b; Eken et al. 1989; Harvey et al. 2006; Li et al. 2007). The recovery of PICs has been linked to the recovery of lost functions, such as locomotion (Fouda et al. 2010; Murray et al. 2010), but also to the development of hyperreflexia (Bennett et al. 1999, 2001a, 2001b) in animal models.

An indirect method that was proposed to evaluate neuronal PICs consists of providing successive stimuli to elicit temporal facilitation of reflex responses, termed wind-up or warm-up (Bennett et al. 1998; Garrison et al. 2011; Hornby et al. 2003, 2006; Onushko et al. 2011; Svirskis and Hounsgaard 1997), a phenomenon originally defined in the spinal cord over 50 years ago (Mendell and Wall 1965). Although most studies to date have primarily focused on wind-up in dorsal horn interneurons or root reflexes in response to nociceptive stimulation (reviewed in Herrero et al. 2000), a few studies have used wind-up of reflexes as an indirect indicator of motoneuronal PICs in an SCI context (Garrison et al. 2011; Hornby et al. 2003, 2006; Onushko et al. 2011).

In these studies it was proposed that wind-up of reflex responses emerged only after SCI, reflecting abnormal regulation within neuronal circuits, thus contributing to the development of spasticity and hyperreflexia (Garrison et al. 2011; Hornby et al. 2006). However, if reflex wind-up is mediated by neuronal PICs, then it should also be present when the spinal cord is intact due to monoaminergic drive from the brain stem. Garrison et al. (2011) attempted to elicit wind-up of stretch

Address for reprint requests and other correspondence: A. Frigon, Université de Sherbrooke, 3001, 12e Ave. Nord, Dept. of Physiology and Biophysics, Faculty of Medicine and Health Sciences, Sherbrooke, QC, Canada J1H 5N4 (e-mail: alain.frigon@usherbrooke.ca).
reflexes in intact rats but could not conclusively validate or exclude its presence due to a large amount of activity triggered by the stimuli. In human experiments, reflex testing is generally done with instructions to relax completely (i.e., at rest) or with a tonic contraction, both of which modify descending control of spinal excitability. Descending commands from supraspinal structures can exert potent actions on spinal reflexes (Eccles and Lundberg 1958; Holmqvist and Lundberg 1961; Hultborn 2001; Lundberg 1964), and relaxing completely or maintaining a tonic contraction changes the level of spinal excitability.

In the present study, we evaluated the presence of reflex wind-up when the spinal cord was intact in decerebrate cats, thus removing the voluntary component. Reflex wind-up was evoked by repetitive electrical stimulation of sensory afferents from the foot, as done previously in human spinal cord-injured subjects (Hornby et al. 2003). The next objective was to evaluate if reflex wind-up persisted following an acute spinal transection. As stated, studies using intracellular recordings have shown that motoneuronal PICs are abolished following an acute spinal transection in isolated rat spinal cord preparations (Bennett et al. 2001b) and in decerebrate cats (Crone et al. 1988; Hounsgaard et al. 1988; Hyngstrom et al. 2008). We hypothesized that reflex wind-up would be present in decerebrate cats when the spinal cord is intact and considerably reduced following acute spinalization, consistent with a descending control of PICs. Although the decerebration can modify descending inputs to the spinal cord (Crone et al. 1988), a decerebrate preparation is required to evaluate the effect of acute spinal transection in the absence of anesthetics.

The type of stimulation that we used also provided the opportunity to evaluate the impact of acute spinalization on two different components of the evoked reflex. Before the acute spinal transection, electrical pulses evoked two distinct signals: a short-latency (≈10 ms) compound action potential (CAP) that was followed by a sustained burst of activity (SA). These potentials were measured separately because they showed distinct modulation patterns following acute spinalization.

Wind-up of reflexes could be a powerful tool to study functional changes in spinal neuronal excitability in response to various treatments in human subjects. However, parallel experiments in animals are necessary to validate the methodology and determine some of the underlying neuronal mechanisms involved in the modulation of reflex wind-up in various injury-induced states. The present results provide a novel technique to assess reflex wind-up evoked before and after an acute spinal transection in the same animals. Such results will serve to guide future intracellular experiments and long-term studies using chronic implantation procedures.

**MATERIALS AND METHODS**

**Ethical information and surgical procedures.** All procedures were approved by the Institutional Animal Care and Use Committee of Northwestern University. All animals were obtained from a designated breeding establishment for scientific research. Before the experiments, animals were housed and fed within designated areas, which were monitored daily by veterinarians and trained personnel. The current data set is compiled from 9 adult cats weighing between 2.5 and 5.0 kg.

Cats were first placed in a clear plastic cylinder and anesthetized with 1.5–3% isoflurane in a 1:3 mixture of O2 and N2O. After ≈15 min, the animal was transferred from the cylinder to a surgical table and anesthesia was continued with a mask. Once the animal was deeply anesthetized, a tracheostomy was performed and cats were intubated to deliver the anesthesia. The right common carotid artery and right jugular vein were cannulated to monitor blood pressure and for fluid administration, respectively. The level of anesthesia was confirmed and adjusted by monitoring blood pressure, applying pressure to the paw to detect limb withdrawal, and by verifying the size and reactivity of the pupils. The animal was then transferred to a stereotaxic frame. A small laminectomy was made at the junction between the 12th and 13th thoracic vertebrae for the acute spinal transection. The dura was removed to expose the spinal cord. After a craniotomy was performed, the cortex and all neural tissue rostral to the colliculi were removed (i.e., a precollucicular decerebration). At this point, animals are considered to have complete lack of sentence (Silverman et al. 2005) and anesthesia was discontinued. A lethal injection of potassium chloride (2 mg/kg) was administered at the end of the experiment through the right jugular vein.

**Experimental design.** A schematic illustration of the experimental setup is shown in a recent publication (Frigon et al. 2012). Both hind paws were held with a clamp. The left knee joint was also fixed with a custom-made clamp attached to the femoral epicondyles. Hip, knee, and ankle joint angles were ≈120°, 160°, and 90° for both hindlimbs. The hind paws were not contacting the table surface.

Bipolar wire electrodes were inserted for electromyography (EMG) into the soleus (ankle extensor), lateral gastrocnemius (LG; ankle extensor/knee flexor), semitendinosus (St; knee flexor/hip extensor), anterior sartorius (Srt; hip flexor/knee extensor), and tibialis anterior (TA; ankle flexor) of the left hindlimb and in the right St. Only data from the left LG, TA, St, and Srt are reported in this study. EMG signals were amplified (×1,000) with a multichannel amplifier (AM Systems, model 3500), bandpass filtered (300–3,000 Hz), and sampled at 10,000 Hz. Bipolar stimulating cuff electrodes were placed around the left tibial (Tib) and superficial peroneal (SP) nerves near the ankle joint in 9 and 6 cats, respectively.

**Experimental protocol.** Data acquisition started no less than 2 h following decerebration. Motor thresholds for nerve stimulation were first determined. For the Tib nerve, the motor threshold was the stimulation intensity required to evoke a small plantar flexion of the left toes. The motor threshold for SP nerve stimulation was the stimulation intensity required to evoke a small flexion response at the knee. Stimulation intensity is expressed as multiples of these thresholds (T). A series of 10 pulses (0.2-ms pulse width) to the left Tib or SP nerves was given at 2T or 5T at a stimulation frequency of 1 or 2 Hz. Wind-up of reflex responses is more evident at stimulation frequencies of 1 Hz or more (Hornby et al. 2003). Generally, two trials were averaged for each stimulation parameter. Nerve stimulations were performed at intervals of no less than 1 min.

After data were obtained in the spinal-intact state, gaseous anesthesia was remixed for ≈5 min to facilitate spinal transection. Lidocaine (Xylocaine, 2%) was applied topically to the exposed spinal segment. After a few minutes, the spinal cord was completely transected with surgical scissors and anesthesia was discontinued. A large powerful involuntary contraction of back and hindlimb muscles is sometimes produced during an acute spinalization if gaseous anesthesia and lidocaine are not used. Reflex testing resumed no less than 1 h following the acute spinal transection.

**Measurements.** All EMG measurements were done with Spike2 version 6.0 and are illustrated in Fig. 1. Two EMG measures were made. The first measure was the short-latency CAP (Fig. 1B), a signal that primarily results from synchronous activation of multiple motor units. To calculate the short-latency CAPs, 10 windows were placed following each pulse from 0.006 to 0.031 s to encompass the short-latency excitatory response, which is evoked at ≈−8 to −10 ms following stimulation of cutaneous nerves at rest or during locomotion in intact
Fig. 1. Measurements of reflex responses. Example shown was obtained by stimulating the tibial nerve (Tib n.) at 5 times threshold (5T) with a frequency of 1 Hz. A: to measure the amplitude of sustained activity (SA), the electromyogram (EMG) waveforms were rectified and then smoothed with a 0.03-s time constant. A moving window was then generated with cursor 1 placed 0.05 s after the stimulation pulse and cursor 2 at the next stimulation pulse. Ten such windows were made to measure SA amplitude following each stimuli (D). The area under the rectified-smoothed curve was calculated using the Modulus function in Spike2 version 6.0. B: the short-latency compound action potential (CAP) was calculated as the peak-to-peak amplitude from the maximum and minimum values obtained within a window from 0.005 to 0.031 s following each stimulation pulse (C). E: the first 5 data points were normalized to the first stimulation pulse, and a linear regression analysis was made to measure the slope ($b[1]$) and the coefficient of determination ($r^2$). St, semitendinosus.
or spinalized cats (Frigon and Rossignol 2007, 2008a, 2008b). The minimal and maximal values were determined within these windows and the peak-to-peak amplitude was measured (in mV).

The second EMG measure was made to assess the presence of sustained activity (SA) following stimulation. The SA is an asynchronous signal that generally takes the form of a burst of activity poststimulation (see Fig. 1A), at least before acute spinal transection. To calculate the SA, the EMG waveforms were rectified and smoothed with a 0.03-s time constant. Ten windows were placed following each pulse from 0.05 to 1 or 0.5 s for 1- and 2-Hz stimulation frequencies, respectively. The SA amplitudes were calculated as the areas under the curve (modulus function in Spike2) within these 10 windows (in mV·s).

To evaluate the presence of wind-up, defined as a progressive increase in response amplitude with successive stimuli (i.e., temporal summation), the slope of the responses evoked by the first five stimulations was calculated before and after acute spinalization (n = 9). To facilitate comparisons before and after acute spinal transection, the first five data points were normalized to the first response [Fig. 1E; see also (Hornby et al. 2003)]. Only the first five values were used because we observed that responses tended to plateau around the fifth stimulation (e.g., Fig. 1D), as shown previously in human spinal cord-injured subjects (Hornby et al. 2003, 2006; Onushko et al. 2011). Slopes and coefficients of determination ($r^2$) were measured by linear regression analysis using SigmaPlot 11.0 (Systat Software). Slope calculations have been used to assess the presence of wind-up (Mitsuyu et al. 2006).

**Results**

The first objective was to determine the effects of stimulation intensity, stimulation frequency, and acute spinalization on the wind-up of reflex responses evoked by a series of 10 pulses. The second objective that emerged from the data set was to evaluate if the first five stimulation pulses was calculated before and after acute spinalization (n = 9). To facilitate comparisons before and after acute spinal transection, the first five data points were normalized to the first response [Fig. 1E; see also (Hornby et al. 2003)]. Only the first five values were used because we observed that responses tended to plateau around the fifth stimulation (e.g., Fig. 1D), as shown previously in human spinal cord-injured subjects (Hornby et al. 2003, 2006; Onushko et al. 2011). Slopes and coefficients of determination ($r^2$) were measured by linear regression analysis using SigmaPlot 11.0 (Systat Software). Slope calculations have been used to assess the presence of wind-up (Mitsuyu et al. 2006).

**Statistical analyses**

Statistical tests were performed with SPSS 18.0 (IBM). Data obtained by stimulating the left Tib and SP nerves were treated separately. To evaluate the effect of stimulation pulse, stimulation intensity, stimulation frequency, and acute spinal transection, we performed a four-factor repeated-measures analysis of variance (ANOVA). The four-factor repeated-measures ANOVA was conducted independently for CAP and SA responses evoked in LG, TA, St, and Srt for Tib and SP nerve stimulations for a total of 16 tests. We also performed a three-factor repeated-measures ANOVA on the slope of the first five stimulation values to evaluate the effect of stimulation intensity, stimulation frequency, and acute spinal transection. The three-factor repeated-measures ANOVA was conducted independently for CAPs and SAs evoked in LG, TA, St, and Srt for Tib and SP nerve stimulations for a total of 16 tests. Bonferroni corrections for multiple tests were not performed (see Rothman 1990 for a debates of this issue). Statistical significance was set at $P \leq 0.05$.

**Results**

The first objective was to determine the effects of stimulation intensity, stimulation frequency, and acute spinalization on the wind-up of reflex responses evoked by a series of 10 pulses. The second objective that emerged from the data set was to evaluate if the amplitude of synchronous (i.e., CAP) and asynchronous (i.e., SA) electrically evoked motor activities showed similar or different patterns of modulation following acute spinal transection.

**Effect of stimulation intensity, frequency, and acute spinalization on the wind-up of reflex responses.** Figure 2 shows examples from one cat with stimulation of the left Tib nerve at 2T and 5T, at 1 and 2 Hz, before (left) and after (right) acute spinalization. In the spinal-intact state (i.e., before the acute spinal transection), each pulse evoked bursts of activity that displayed wind-up in the three muscles shown for the first three to four stimulation pulses. In the acute spinalized state (i.e., after the acute spinal transection), these bursts were reduced and displayed no visible evidence of wind-up. In the example shown, bursts evoked at 5T were generally larger than those evoked at 2T, primarily in the spinal-intact state. The effect of frequency on the bursting was also more apparent in the spinal-intact state, with more sustained activity in between pulses at 2 Hz. Overall activity was diminished following acute spinal transection. However, the short-latency CAPs were increased, particularly in St and TA. To better demonstrate changes in short-latency CAPs, Fig. 3 shows the same EMG waveforms as in Fig. 2 with a 50-ms window that follows the fifth stimulation pulse in each condition. As can be seen, the short-latency CAPs that followed the stimulation with a latency of ~8–10 ms were increased after acute spinal transection, particularly for St and TA, irrespective of stimulation parameters.

Figures 4 and 5 show reflex responses evoked by stimulating the left Tib and SP nerves, respectively, with a series of 10 stimulation pulses at 2T and 5T, at 1 and 2 Hz, for the group. In Figs. 4 and 5, left and right panels show the amplitudes of the CAPs and SAs, respectively. Error bars are not shown because of the number of data points. A four-factor repeated-measures ANOVA was conducted for CAPs and SAs for each muscle and nerve stimulation to determine the effect of stimulation pulse, stimulation intensity, stimulation frequency, and acute spinal transection on reflex responses (see METHODS). Overall, Figs. 4 and 5 show that reflex responses display wind-up (i.e., an increased response amplitude with successive stimuli) in most muscles, particularly before the acute spinal transection (i.e., with an intact spinal cord).

For CAP amplitudes evoked by stimulating the Tib nerve, there was a significant effect of stimulation intensity in TA only (Fig. 4C). The CAP responses evoked at 2T in TA before the acute spinal transection were smaller than at 5T. There was no significant effect of stimulation frequency on CAPs evoked by Tib nerve stimulation in any of the recorded muscles. Acute spinal transection had a significant effect on CAP responses in TA (Fig. 4C) and St (Fig. 4E). As can be seen, CAP responses evoked in TA and St were generally increased after acute spinal transection. For SA responses evoked by stimulating the Tib nerve, there was a significant effect of stimulation intensity in TA (Fig. 4D) and St (Fig. 4H), with responses evoked at 5T before acute spinal transection being larger than those evoked at 2T. There was a significant effect of frequency on SA responses evoked in St only (Fig. 4H). Acute spinal transection significantly influenced SA responses in TA only, with responses being smaller after acute spinalization (Fig. 4D).

For CAP responses evoked by stimulating the SP nerve, there was a significant effect of stimulation intensity in LG (Fig. 5A) and TA (Fig. 5C). As can be seen, CAP responses were consistently larger at 5T compared with those evoked at 2T in LG and TA. There was an effect of stimulation frequency on CAPs evoked by SP nerve stimulation in St only (Fig. 5E). This effect was most evident before the acute spinalization. Acute spinal transection had a significant effect on CAP responses in TA (Fig. 5C), St (Fig. 5E), and Srt (Fig. 5G). In all three muscles, CAP amplitudes were larger after acute spinalization. For SA responses evoked by SP nerve stimulation, there was an effect of stimulation intensity in St only (Fig. 5F), with responses evoked at 5T being consistently larger than those evoked at 2T before and after acute spinal transection. There was no effect of stimulation frequency on SA responses in any of the recorded muscles. Acute spinal transection had an effect on SA responses in TA only, and again, responses were smaller after acute spinalization (Fig. 5D).

J Neurophysiol • doi:10.1152/jn.00683.2012 • www.jn.org
Fig. 2. State-dependent modulation of reflex responses evoked during a series of 10 stimulation pulses to the Tib nerve in 1 cat. The example shows a series of 10 stimulation pulses to the left Tib nerve at 2T and 5T at 1 and 2 Hz in cat 71511 before (spinal-intact) and after (acute spinalized) the acute spinal transection. Note that the vertical scale is the same for a given muscle in all graphs, but the horizontal scale differs for 1- and 2-Hz stimulation. TA, tibialis anterior; LG, lateral gastrocnemius.
To quantify the presence of wind-up, we calculated the slope of the first five responses evoked by stimulating the Tib and SP nerves at 2T and 5T, at 1 and 2 Hz, before and after acute spinal transection (see METHODS). A three-factor repeated-measures ANOVA was conducted for CAPs and SAs for each muscle and nerve stimulation to determine the effect of stimulation intensity, stimulation frequency, and acute spinal transection on reflex wind-up (see METHODS). There were no significant effects of stimulation intensity or frequency on the slope of reflex responses evoked by Tib nerve stimulations in any of the recorded muscles. However, acute spinal transection significantly affected wind-up of CAPs in Srt and SA responses in St and TA. Because there were no significant effects of stimulation intensity or frequency, data were pooled and pairwise comparisons were performed (Fig. 6A). For reflex responses evoked by stimulating the SP nerve, stimulation intensity significantly influenced slope values for CAP responses evoked in LG and TA and for SA responses evoked in St. Frequency significantly influenced CAP responses in St. Acute spinal transection influenced SA responses in St only. We found no significant interaction between stimulation intensity and state for SA responses in St ($P = 0.11$), so data were pooled and pairwise comparisons were performed (Fig. 6B). For reflex responses that were influenced by acute spinalization, in each case, acute spinal transection significantly reduced the slope of the first five reflex responses (Fig. 6). These data suggest that reflex wind-up is significantly reduced after acute spinal transection in certain muscles.

**DISCUSSION**

The present study showed temporal summation of reflex responses evoked by stimulating afferents from the foot when the spinal cord was intact, which was reduced in some muscles after acute spinal transection in decerebrate cats. In addition, synchronous and asynchronous signals evoked by the same electrical stimuli showed marked differences in their modulation patterns in some muscles following acute spinalization. The decerebrate cat preparation constitutes a novel technique to study the wind-up of reflex responses in the intact spinal cord.

Effect of stimulation intensity, frequency, and acute spinal transection on reflex responses. Statistical analyses revealed that stimulation intensity influenced at least one reflex response (CAP or SA) in three of four recorded muscles (i.e., LG, TA, and St), whereas stimulation frequency only influenced reflex responses in St. Acute spinal transection influenced at least one reflex response in Srt, TA, and St. Thus reflex responses evoked in LG, the only extensor muscle, were not affected by acute spinalization, as opposed to muscles that perform flexion at the hip, knee, or ankle joints. The existence of alternate reflex pathways within the spinal cord, which are regulated by descending pathways and other segmental inputs (Holmqvist and Lundberg 1961; Hultborn 2001; Jankowska and Hammar 2002; Lundberg 1964) are most likely responsible for differential changes in reflex responses in flexor and extensor muscles after acute spinal transection (see also Frigon et al. 2011).

Reflex responses evoked in our study were most likely mediated by polysynaptic pathways activated by cutaneous inputs. Although responses evoked by stimulating the Tib nerve at the ankle are often ascribed to cutaneous afferents
sensory inputs from mechanoreceptors of intrinsic foot muscles cannot be excluded. However, reflex responses evoked by stimulating afferents from the SP nerve, which are entirely cutaneous at the ankle (Bernard et al. 2007), showed patterns of change similar to those evoked by the Tib nerve stimulation following acute spinalization, indicating that the observed effects were primarily mediated by cutaneous inputs. Stimulation at 2T mostly recruits large-diameter cutaneous afferents, whereas 5T stimulation likely activates Aδ afferents as well. The significant effect of stimulation intensity on some reflex responses (Figs. 4 and 5) could be due to greater recruitment of large-diameter fibers. Further investigations are required, however, to determine the relative contribution of different types of afferents in CAP and SA responses.

(Frigon and Rossignol 2008a; Loeb 1993), sensory inputs from mechanoreceptors of intrinsic foot muscles cannot be excluded. However, reflex responses evoked by stimulating afferents from the SP nerve, which are entirely cutaneous at the ankle (Bernard et al. 2007), showed patterns of change similar to those evoked by the Tib nerve stimulation following acute spinalization, indicating that the observed effects were primarily mediated by cutaneous inputs. Stimulation at 2T mostly recruits large-diameter cutaneous afferents, whereas 5T stimulation likely activates Aδ afferents as well. The significant effect of stimulation intensity on some reflex responses (Figs. 4 and 5) could be due to greater recruitment of large-diameter afferents and/or the additional recruitment of Aδ fibers. Further investigations are required, however, to determine the relative contribution of different types of afferents in CAP and SA responses.

Fig. 4. Reflex responses evoked by a series of 10 stimulation pulses to the Tib nerve for the group. A, C, E, and G show the amplitudes of the short-latency CAP, and B, D, F, and H show the SA amplitudes, for the 4 muscles before (spinal-intact, INT) and after (spinal-transected, ST) acute spinalization at 2T and 5T, at 1 and 2 Hz. The data points in LG, TA, St, and anterior sartorius (Srt) are the average of 5, 8, 6, and 8 cats, respectively. Because of noise in some recordings, not all muscles and stimulation parameters were available for all cats.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Stimulation Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>LG</td>
<td>INT - 2T, 1Hz</td>
</tr>
<tr>
<td></td>
<td>INT - 2T, 2Hz</td>
</tr>
<tr>
<td></td>
<td>INT - 5T, 1Hz</td>
</tr>
<tr>
<td></td>
<td>INT - 5T, 2Hz</td>
</tr>
<tr>
<td>TG</td>
<td>ST - 2T, 1Hz</td>
</tr>
<tr>
<td></td>
<td>ST - 2T, 2Hz</td>
</tr>
<tr>
<td></td>
<td>ST - 5T, 1Hz</td>
</tr>
<tr>
<td></td>
<td>ST - 5T, 2Hz</td>
</tr>
</tbody>
</table>

CAP amplitude (mV)
SA amplitude (mV/s)

<table>
<thead>
<tr>
<th>Stimulation pulse</th>
<th>LG - Int 2T, 1Hz</th>
<th>LG - Int 2T, 2Hz</th>
<th>LG - Int 5T, 1Hz</th>
<th>LG - Int 5T, 2Hz</th>
<th>LG - ST 2T, 1Hz</th>
<th>LG - ST 2T, 2Hz</th>
<th>LG - ST 5T, 1Hz</th>
<th>LG - ST 5T, 2Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>2</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.13</td>
<td>0.13</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>3</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>4</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>5</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.12</td>
<td>0.12</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>6</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>7</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stimulation pulse</th>
<th>TA - Int 2T, 1Hz</th>
<th>TA - Int 2T, 2Hz</th>
<th>TA - Int 5T, 1Hz</th>
<th>TA - Int 5T, 2Hz</th>
<th>TA - ST 2T, 1Hz</th>
<th>TA - ST 2T, 2Hz</th>
<th>TA - ST 5T, 1Hz</th>
<th>TA - ST 5T, 2Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>3</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>4</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>5</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>6</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>7</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stimulation pulse</th>
<th>St - Int 2T, 1Hz</th>
<th>St - Int 2T, 2Hz</th>
<th>St - Int 5T, 1Hz</th>
<th>St - Int 5T, 2Hz</th>
<th>St - ST 2T, 1Hz</th>
<th>St - ST 2T, 2Hz</th>
<th>St - ST 5T, 1Hz</th>
<th>St - ST 5T, 2Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>3</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>4</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>5</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>6</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>7</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stimulation pulse</th>
<th>Srt - Int 2T, 1Hz</th>
<th>Srt - Int 2T, 2Hz</th>
<th>Srt - Int 5T, 1Hz</th>
<th>Srt - Int 5T, 2Hz</th>
<th>Srt - ST 2T, 1Hz</th>
<th>Srt - ST 2T, 2Hz</th>
<th>Srt - ST 5T, 1Hz</th>
<th>Srt - ST 5T, 2Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>3</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>4</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>5</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>6</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

J Neurophysiol • doi:10.1152/jn.00683.2012 • www.jn.org
Differential modulation of synchronous vs. asynchronous signals. As already stated, electrical stimulation evoked two distinct components of the reflex responses based on their modulation pattern following acute spinalization, suggesting that they are mediated by different neuronal mechanisms. Specifically, when acute spinal transection significantly influenced CAP and SA responses, short-latency CAPs were increased following acute spinal transection, whereas SA responses were generally decreased (see Figs. 4 and 5). CAPs are most likely generated by the synchronous activation of several excitatory interneurons that in turn activate multiple motoneurons through fast glutamatergic synaptic transmission. In a similar vein, intracellular studies showed that electrically evoked short-latency homonymous and heteronymous excitatory signals differ in their modulation patterns following acute spinal transection.

Fig. 5. Reflex responses evoked by a series of 10 stimulation pulses to the superficial peroneal (SP) nerve for the group. A, C, E, and G show the amplitudes of the short-latency CAP, and B, D, F, and H show the SA amplitudes, for the 4 muscles before (INT) and after (ST) acute spinalization at 2T and 5T, at 1 and 2 Hz. The data points in LG, TA, St, and Srt are the average of 5, 5, 3, and 5 cats, respectively. Because of noise in some recordings, not all muscles and stimulation parameters were available for all cats.
In the spinal-intact state, sustained activity at the muscle following stimulation of cutaneous afferents from the foot was most likely mediated by the activation of intrinsic neuronal properties that in turn produced asynchronous discharges in multiple interneurons and motoneurons. The reduction in wind-up after acute spinal transection was most evident for SA responses, with two of four muscles (St and TA) showing a significant decrease with Tib nerve stimulation and one of four muscles (St) with SP nerve stimulation (see Fig. 6). Only CAP responses in St evoked by Tib nerve stimulation were influenced by acute spinal transection. These results are consistent with a role for PICs in sustaining neuronal firing. PICs are considerably reduced after acute spinalization in decerebrate cats due to the loss of descending monoaminergic inputs (Crone et al. 1988; Hounsgaard et al. 1988; Hylleberg et al. 2008). Indeed, motoneuronal PICs can be facilitated by exogenous application of serotoninergic (Hounsgaard et al. 1988) and noradrenergic (Conway et al. 1988) agonists in acutely spinalized decerebrate cats, as well as in the isolated sacrocaudal spinal cord preparation of adult rats (Harvey et al. 2006). Smaller neuronal PICs after acute spinal transection probably reduced temporal summation of sensory inputs at interneuronal and motoneuronal levels. Although we favor a role for PICs for changes in SA amplitudes following acute spinal transection, complex changes within excitatory and inhibitory spinal neuronal circuits, modified presynaptic inhibition of sensory afferents, and/or changes in synchronization patterns must also be considered.

**Is wind-up indicative of an underlying spinal pathophysiology?** The results of the present study clearly show the presence of wind-up when the spinal cord was intact (e.g., Fig. 2). It was proposed that wind-up is linked to neuronal dysfunction (e.g., spasticity) because its presence was only reported in spinal cord-injured patients (Garrison et al. 2011; Hornby et al. 2003, 2006). However, in those studies no data from a neurologically intact group were shown and no statistical comparisons were made between intact and SCI groups. In the study by Garrison et al. (2011), an attempt was made to evoke wind-up in intact rats, but they stated that “repeated perturbations produced a dominating voluntary EMG and force activity that prevented assessment of the reflex component.” It is possible that the dominating EMG and force activity was due to wind-up of spinal neuronal activity that engaged movement via a reflex mechanism and not by a voluntary component. The absence of wind-up in neurologically intact subjects reported in the literature is not consistent with a role for neuromodulators released from descending pathways in the control of PICs, which are in high supply when the spinal cord is intact. The present work and that of Bennett et al. (1998) clearly show that wind-up of reflex responses is present in decerebrate cats with an intact spinal cord. Although decerebration can be considered a pathological state that might lead to an increased tonic descending serotonergic drive (Crone et al. 1988), it is clear that wind-up of reflex responses does not emerge strictly after SCI. However, at present, we cannot exclude that wind-up is absent in a fully intact preparation (i.e., not decerebrated) due to descending inhibition of spinal neurons interposed in reflex pathways (Eccles and Lundberg 1958; Holmqvist and Lundberg 1961; Hultborn 2001; Lundberg 1964, 1979), which is reduced or abolished by decerebration or spinal transection.

---

**Fig. 6. Modulation of the wind-up of reflex responses by acute spinalization.** In the 9 cats, for each stimulation parameter (i.e., 2T or 5T at 1 or 2 Hz), the first 5 amplitudes of the short-latency CAP and of the SA were normalized to the 1st response. The slope of the normalized reflex responses was then measured by linear regression analysis. The graph summarizes reflex responses that were significantly affected by acute spinal transection (3-factor repeated-measures ANOVA). ***p < 0.001, significant differences between pooled data from the spinal-intact and acute spinal-transected states (pairwise comparisons).
Functional considerations and concluding remarks. The difference in the pattern of modulation of CAP and SA responses after acute spinal transection indicates that the type of reflex, or component of the reflex, that is being measured must be considered an important factor when determining functional changes in circuitry or overall excitability following SCI or other damage to the nervous system. As stated by Bennett et al. (1998), wind-up of reflex responses may be a functionally important form of short-term plasticity that facilitates motor output once a certain threshold is reached for a certain period of time. Wind-up behavior could be a powerful tool to study neuronal function following SCI, and therapies aimed at regulating wind-up could prove effective in restoring movement and managing spasticity. At present, however, we are limited by our poor knowledge of the evolution of wind-up over time after SCI, which can only be evaluated using chronic implantation procedures in an animal model. Because spasticity takes several weeks to develop, evaluating wind-up concurrently with spasticity in chronically implanted animals could provide important information regarding some of the underlying neurophysiological mechanisms generating spasticity. Intracellular recordings from sensory afferents, interneurons, and motoneurons in the curarized decerebrate cat could also help delineate the contribution of different types of neurons and afferents in the generation of wind-up before and after spinal transection. The decerebrate cat preparation is also amenable to testing and comparing other types of reflex wind-up (e.g., stretch reflexes, H-reflexes) and its pharmacological regulation before and after various types of spinal lesions.

ACKNOWLEDGMENTS

We thank Marin Manuel and Jack Miller for technical assistance.

GRANTS

The present research was funded by an individual grant from the Wings for Life Foundation and by a postdoctoral fellowship from the Canadian Institutes of Health Research (to A. Frigon), as well as by National Institute of Neurological Disorders and Stroke Grant NS034382 (to C. J. Heckman).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


---

*J Neurophysiol* • doi:10.1152/jn.00683.2012 • www.jn.org