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Postactivation depression and recovery of reflex transmission during repetitive electrical stimulation of the human tibial nerve

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Clair JM, Anderson-Reid JM, Graham CM, Collins DF. Postactivation depression and recovery of reflex transmission during repetitive electrical stimulation of the human tibial nerve. *J Neurophysiol* 106: 184–192, 2011. First published April 20, 2011; doi:10.1152/jn.00932.2010.—H-reflexes are progressively depressed, relative to the first response, at stimulation frequencies above 0.1 Hz (postactivation depression; PAD). Presently, we investigated whether H-reflexes "recover" from this depression throughout 10-s trains of stimulation delivered at physiologically relevant frequencies (5–20 Hz) during functionally relevant tasks (sitting and standing) and contraction amplitudes [relaxed to 20% maximum voluntary contraction (MVC)]. When participants held a 10% MVC, reflex amplitudes did not change during 5-Hz stimulation. During stimulation at 10 Hz, reflexes were initially depressed by 43% but recovered completely by the end of the stimulation period. During 20-Hz stimulation, reflexes were depressed to 10% and recovered to 36% of the first response, respectively. This "postactivation depression and recovery" (PAD&R) of reflex amplitude was not different between sitting and standing. In contrast, PAD&R were strongly influenced by contraction amplitude. Reflexes were depressed to 10% of the first response during the relaxed condition (10-Hz stimulation) and showed no depression during a 20% MVC contraction. A partial recovery of reflex amplitude occurred when participants were relaxed and during contractions of 1–5% MVC. Surprisingly, reflexes could recover completely by the third pulse within a stimulation train when participants held a contraction between 5 and 10% MVC during stimulation at 10 Hz, a finding that challenges classical ideas regarding PAD mechanisms. Our results support the idea that there is an ongoing interplay between depression and facilitation when motoneurons receive trains of afferent input. This interplay depends strongly on the frequency of the afferent input and the magnitude of the background contraction but is relatively insensitive to changes in task.

synaptic efficacy; H-reflex; M-wave; neuromuscular electrical stimulation; homosynaptic depression

THE INTEGRATION OF AFFERENT FEEDBACK and motor output is a fundamental component of the neural control of human movement. It is clear that such sensorimotor integration is not hard-wired, but rather depends on many factors, including the frequency of the afferent volley (Ishikawa et al. 1966; Magladery 1955), the task being performed (Capaday and Stein 1986; Hayashi et al. 1992; Krauss and Misiaszek 2007), and the magnitude of the ongoing voluntary contraction (Burke et al. 1989; Stein et al. 2007; Trimble et al. 2000). One of the strongest and most studied afferent projections to motoneurons is that of large-diameter (Ia) afferents from muscle spindles. During natural movements, motoneurons receive trains of impulses from Ia afferents over a range of frequencies (Vallbo 1973), and the efficacy of synaptic transmission depends strongly on impulse frequency (Curtis and Eccles 1960; Lüscher et al. 1983). In humans, H-reflexes are progressively depressed when the frequency of the afferent volley increases above 0.1 Hz (Burke et al. 1989; Goulart et al. 2000; Ishikawa et al. 1966; Stein et al. 2007). This attenuation in reflex transmission, referred to as low-frequency (Ishikawa et al. 1966), homosynaptic (Beswick and Evanson 1957), or postactivation depression (PAD; Crone and Nielsen 1989), is thought to be due to a presynaptic mechanism involving a decreased probability of neurotransmitter release from previously active Ia-afferent terminals (Hirst et al. 1981; Hultborn et al. 1996; Kuno 1964).

The most common way to quantify PAD in humans has been to compare the amplitude of two reflexes evoked over a range of stimulation frequencies (Burke et al. 1989; Crone and Nielsen 1989; Oya and Cresswell 2008; Ruegg et al. 1990; Van Boxtel 1986). Relative to the first response, the second reflex is typically depressed by ~80% at frequencies of 4–10 Hz (Burke et al. 1989; Stein et al. 2007) and can be reduced further at frequencies above 10 Hz (Goulart et al. 2000). An alternative approach has been to deliver a brief train of impulses (up to 30) over a range of frequencies and compare the amplitude of the first reflex to the mean of the subsequent reflexes (Ishikawa et al. 1966; Kohn et al. 1997; Rothwell et al. 1986). Although both of these approaches have provided important information about frequency-dependant depression of reflex transmission, they have not shed light on whether sensorimotor integration along this pathway changes after the initial depression. There is some evidence that soleus H-reflex amplitude recovers partially from the initial depression by the end of a 7-s (20 Hz) stimulation train (Klakowicz et al. 2006). Thus the first goal of the current study was to quantify the postactivation depression and recovery (PAD&R) of transmission along the H-reflex pathway throughout 10-s trains of stimulation delivered over a range of stimulation frequencies.

Despite the strength and ubiquitous nature of PAD, whether it plays a significant role in the neural control of human movement remains controversial. It has been proposed that PAD attenuates afferent transmission to maintain the sensitivity of motoneurons to other synaptic inputs during movement (Hultborn and Nielsen 1998). However, the inability to measure PAD during voluntary contractions and during certain tasks has been cited as evidence that PAD is not a factor when performing functional movements (Stein et al. 2007). PAD was reduced or absent when seated participants held a voluntary contraction (Burke et al. 1989; Floeter and Kohn 1997; Mc-

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Nulty et al. 2008; Oya and Cresswell 2008; Rothwell et al. 1986; Ruegg et al. 1990; Stein et al. 2007; Trimble et al. 2000) and was reduced (Field-Fote et al. 2006; Goulart et al. 2000) or absent (Stein et al. 2007) during standing compared with sitting. As a voluntary contraction increases, the number and discharge rate of active muscle spindles also increase. This increase in afferent discharge invokes PAD at previously silent synapses and enhances PAD at synapses that were already active, with the end result being a tonic depression of synaptic transmission. Thus, when experimental approaches are used to assess PAD during voluntary contractions, the first reflex is evoked at a time when synaptic transmission is already depressed, and the ability to demonstrate any further depression decreases as contraction amplitude increases (Hultborn and Nielsen 1998; Stein et al. 2007). A second goal of the current study was to determine whether changes in PAD that have previously been attributed to task may instead be related to differences in contraction amplitude.

In the present study we quantified the effects of stimulation frequency (5, 10, or 20 Hz), task (sitting vs. standing), and background contraction amplitude [relaxed to 20% maximum voluntary contraction (MVC)] on the PAD&R of soleus H-reflexes during 10-s trains of stimulation. In general, we predicted that reflexes would be significantly depressed immediately after the first reflex but that reflex amplitude would recover from the initial depression over the 10-s stimulus train. Our specific hypotheses related to frequency, task, and contraction level were: *1*) PAD&R depends on stimulus frequency; there will be more depression and less recovery of reflex amplitudes as stimulation frequency increases; *2*) PAD&R will not be influenced by task; there will be no difference between PAD&R of reflex amplitudes between sitting and standing; and *3*) PAD&R depends on the level of background contraction; there will be less depression and more complete recovery of reflex amplitudes as contraction level increases. In addition to quantifying H-reflex amplitudes, we also quantified M-wave amplitudes as a measure of stimulus efficacy. The results of these experiments provide insight into sensorimotor integration along the human H-reflex pathway when motoneurons receive trains of afferent impulses at physiologically relevant frequencies during functionally relevant tasks and contraction levels.

METHODS

Eleven participants with no known neurological impairments (8 men and 3 women; 20–46 yr) took part in this study after providing informed and written consent. The study was conducted in two parts with eight participants involved in each part. The experimental protocols were conducted in accordance with the standards set by the Declaration of Helsinki and were approved by the Health Research Ethics Board at the University of Alberta. Each experimental session lasted ~3 h.

Electromyography. Surface electromyography (EMG) was recorded from the right soleus muscle using disposable bipolar surface EMG electrodes (2.54 cm^2, A10043-P; Vermed Medical, Bellows Falls, VT). The EMG signals were band-pass filtered from 30 to 3,000 Hz and amplified 1,000 times (Neurolog System; Digitimer, Welwyn Garden City, UK). A reference electrode was placed on the tibial plateau of the right leg (10.16 × 2 cm, electrosurgical patient plate, split; 3M Health Care, St. Paul, MN).

Maximum voluntary contractions. At the beginning of each experimental session, participants were instructed to plantarflex their right foot by pushing down in a gas pedal motion against a metal footplate, using only their ankle muscles, until they reached their maximum and to hold this contraction for 1–2 s. Participants practiced this action and then performed two to three MVCs until two of their attempts were within 10% of each other. During all MVC trials, the experimenters provided verbal encouragement to the participants to perform maximally.

Nerve stimulation. Electrical stimulation was delivered to the tibial nerve in the right popliteal fossa through disposable bipolar surface EMG electrodes (2.54 cm^2, A10043-P; Vermed Medical) using a constant-current stimulator (DS7A; Digitimer). Each stimulus train was delivered for 10 s (1-ms pulse width) at 5, 10, or 20 Hz. Each trial consisted of three identical stimulation trains separated by 30 s. A 2-min rest period was incorporated between each trial to minimize muscular fatigue. Stimulation intensity was set at the beginning of each trial to evoke a motor wave (M-wave) of ~5% of the maximum M-wave (M_{max}) in response to three single pulses delivered ~5 s apart. Data for soleus M-wave/H-reflex recruitment curves were collected in each experiment (n = 50 stimuli; 1-ms pulse width; 5- to 7-s interstimulus interval) while the participant was seated with the soleus relaxed. Stimulation delivery and data collection were controlled by custom-written software programs (LabView; National Instruments, Austin, TX). All data were sampled at 5,000 Hz and stored on a computer for later analysis.

Part 1 protocol: effects of stimulation frequency and task. Part 1 of this study was designed to assess the effects of stimulation frequency (5, 10, and 20 Hz) and task (sitting and standing) on the PAD&R of soleus H-reflexes. For the standing trials, participants stood with their feet hip width apart, hands at their sides, and looked straight ahead. For the seated trials, subjects sat on the chair of a Biodex dynamometer (System 3; Biodex Medical Systems, Shirley, NY) with their knee and ankle at 110° and 90°, respectively, and also looked straight ahead. While sitting, the participants maintained a background contraction in soleus to match the EMG measured during standing. Visual feedback of the low pass-filtered (3 Hz) soleus EMG signal was displayed on a computer screen to help the seated participants hold the desired level of activity. The stimulation trials included all combinations of frequency (5, 10, and 20 Hz) and task (sitting and standing). Thus there were six stimulation trials, and these were delivered in a random order across participants.

Part 2 protocol: effect of background contraction levels in soleus during sitting. Part 2 of this study was designed to assess the effect of different levels of background contraction on the PAD&R of soleus H-reflexes. Each participant was seated (as described above) and received 10-s trains of stimulation at 10 Hz while they were relaxed or holding a 1, 5, 10, or 20% MVC soleus contraction. Visual feedback of the soleus EMG (as described above) was provided to help the participants maintain the desired contraction.

Data analysis. Data analysis was performed post hoc using custom-written MATLAB software (The MathWorks, Natick, MA). The average over a 500-ms window centered around the peak filtered (low pass, 40 Hz) and rectified EMG in the largest MVC trial was used to calculate the soleus MVC. Background contraction levels were quantified by measuring the filtered (low pass, 40 Hz) and rectified EMG over a 1-s period, centered around 1 s before the stimulation trains in each trial, and normalizing these values to the soleus MVC. The largest M-wave amplitude measured from the recruitment curve trial was considered to be M_{max}. The peak-to-peak amplitude of each M-wave and H-reflex evoked during each stimulus train was measured and then normalized to M_{max}.

To generate group mean M-wave and H-reflex amplitudes, the first (M_1 or H_1) and second (M_2 and H_2) responses for a given condition and participant were averaged separately over the three stimulation trains in each trial (see Fig. 1). In addition, after the first response in each stimulation train, all subsequent responses were averaged over 0.5-s intervals to generate 20 data bins. Data bins calculated for each stimulation train in this way were averaged across the three stimula-

tion trains in each trial. As shown in Fig. 1, one measure was used to quantify the initial reflex depression (PAD), and three measures were used to quantify the reflex recovery. To quantify PAD, the amplitude of the second response was compared with that of the first response. To characterize the "fast" recovery of reflex amplitude that occurred within the first 0.5 s of the stimulus train, the amplitude of the second response was compared with the mean of *bin 1*. To characterize the "slow" recovery that often occurred over the duration of the 10-s stimulation, *bin 1* was compared with *bin 20*. To determine whether the recovery of reflex amplitude was "complete" (i.e., returned to the amplitude of the first response), *bin 20* was compared with the mean amplitude of the first response. In *part 2* of this study, the fast recovery was also investigated with a greater temporal resolution by comparing the amplitudes of the responses evoked by the first six stimulation pulses in each stimulus train.

Statistical analysis. A one-way repeated-measures analysis of variance test (rmANOVA) was used to compare the levels of background contraction between sitting and standing for the experiments described in *part 1* of this study. A one-way rmANOVA was also used to compare the different levels of background contraction in *part 2*. To assess whether the amplitude of the M-waves influenced the amplitude of the H-reflexes during the 10-s trains of stimulation, we used repeated-measures analysis of covariance tests (rmANCOVAs). The difference between the first and second M-wave in each condition (i.e., sitting at 5, 10, and 20 Hz; standing at 5, 10, and 20 Hz; background contraction when relaxed and at 1, 5, 10, and 20% MVC) was used as a covariate in a simplified rmANCOVA conducted to assess changes between the first and the second H-reflexes in each condition, respectively. The covariate was not significant in any of the conditions. In other words, changes in the M-wave amplitude did not significantly account for changes in the H-reflex amplitude in any condition, and thus the M-wave and H-reflex data were analyzed using separate rmANOVAs.

To assess PAD&R in *part 1*, a three-way rmANOVA was used to test for significant effects of stimulation frequency (5, 10, 20 Hz), task (sitting and standing), and time (first response, second response, *bin 1*, *bin 20*) on response amplitude. For *part 2*, a two-way rmANOVA was used to assess the influence of background contraction (relaxed, 1, 5, 10, 20% MVC) and time (first response, second response, *bin 1*, *bin 20*) on response amplitude. To test for changes in H-reflex amplitude over the first six responses in *part 2*, a two-way rmANOVAs were used with background contraction (relaxed, 1, 5, 10, 20% MVC) and time (responses 1–6) as factors. Tukey's honestly significant difference tests were performed when appropriate on significant interactions or main effects identified by the rmANOVA analyses. The alpha level for all statistical analyses was set at 0.05. Descriptive statistics are means ± SD.

RESULTS

We investigated the effects of stimulation frequency, task, and background contraction on the depression and recovery of soleus H-reflexes throughout 10-s trains of electrical stimulation. M-waves were also quantified as a measure of stimulus efficacy. Data from a single participant are shown in Fig. 2 for one 10-s train of 10-Hz stimulation delivered when the participant was seated and holding a background soleus contraction of ~15% MVC. In Fig. 2, *top*, the amplitudes of M-waves and H-reflexes evoked by each stimulus pulse are indicated by open circles and filled diamonds, respectively. Soleus EMG recorded at the beginning and end of the stimulation train is shown in Fig. 2, *bottom*. This participant showed depression of both the M-wave and the H-reflex from the first to the second stimulus pulses. Although M-waves remained stable for the duration of the stimulation, H-reflex amplitude recovered. As early as the third stimulus pulse (200 ms after the first pulse), reflex amplitude had recovered from 19 to 84% of H_1. Throughout the stimulation, reflex amplitude varied, but there was a trend for a slow recovery of reflex amplitude over the 10 s, ending with the last H-reflex being larger than the first H-reflex (108% of H_1).

Part 1: effects of task and stimulation frequency. The group data for all combinations of stimulation frequency and task across the full 10-s stimulation period are shown in Fig. 3. In general, there was more H-reflex depression with 20-Hz stimulation, more H-reflex recovery with 10-Hz stimulation, and no differences in PAD&R between sitting and standing. Soleus background contraction levels were not different between sitting (12 ± 4% MVC) and standing trials (11 ± 4% MVC) [$F_{(1,7)} = 0.84$, $P > 0.1$] (data not shown). For M-wave amplitude, there was a main effect of frequency [$F_{(2,14)} = 8.54$, $P < 0.001$] and no main effects of task or time. The main effect of frequency, with the data collapsed across task and time, revealed a general depression of M-wave amplitude during 10 ($P < 0.001$)- and 20-Hz stimulation ($P < 0.05$), compared with 5-Hz stimulation (data not shown).

The analysis of the depression of H-reflex amplitude identified a significant interaction between frequency (5, 10, and 20 Hz) and time (H_1, H_2, *bin 1*, *bin 20*) [$F_{(6,42)} = 18.93$, $P < 0.001$] and no significant effect of task. The results of the post hoc analysis performed on this interaction are shown in Fig. 4. Depression of reflex amplitude occurred during 10- and 20-Hz

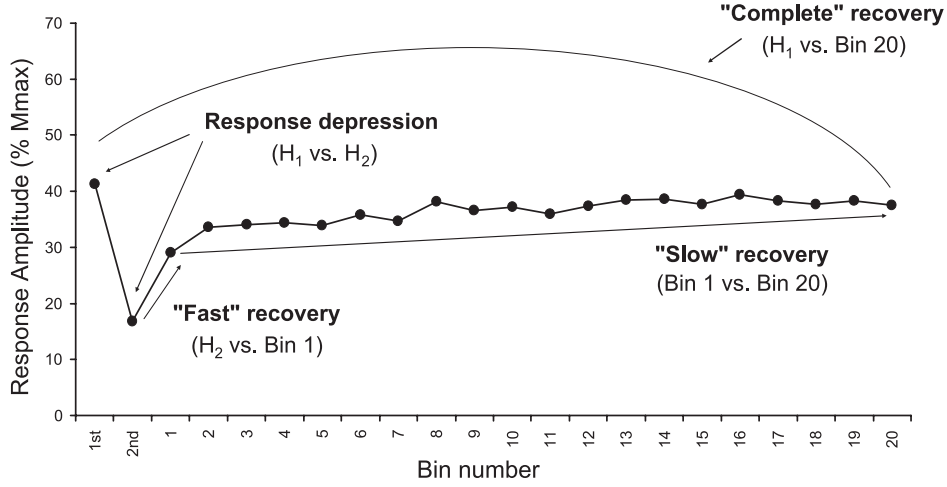

Fig. 1. Diagram illustrating the method used to quantify reflex depression and recovery during 10-s trains of stimulation. The initial depression was assessed by comparing the first (H_1) and second (H_2) reflexes. "Fast" recovery was assessed by comparing H_2 and *bin 1* (responses averaged over the first 0.5 s). "Slow" recovery was assessed by comparing *bin 1* with *bin 20* (responses averaged over the last 0.5 s). "Complete" recovery was assessed by comparing *bin 20* with H_1.

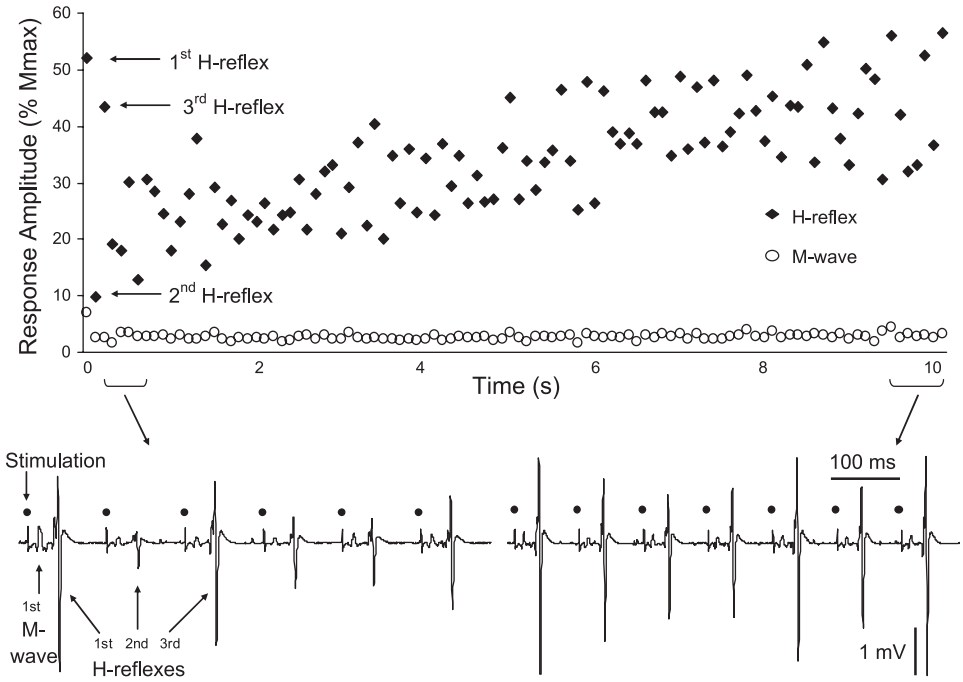

Fig. 2. Data from a single participant during one 10-s train of 10-Hz stimulation, delivered when the participant was seated and holding a background soleus contraction of ~15% maximum voluntary contraction (MVC). *Top*: M-waves and H-reflexes evoked by each stimulus pulse. M_{max}, maximum M-wave. *Bottom*: raw soleus electromyogram (EMG) recorded at the beginning and end of the stimulation train (filled circles denote stimulation artifacts).

stimulation only. There was a significant difference between H_1 and H_2 at 10 (H_2 was 43% of H_1; $P < 0.001$) and 20 Hz (H_2 was 10% of H_1; $P < 0.001$), but not at 5 Hz. H_2 at 20 Hz was also significantly smaller than H_2 at 10 Hz ($P < 0.001$), as denoted by the cross symbols in Fig. 4, indicative of greater depression during 20-Hz stimulation.

The post hoc analysis of the frequency by time interaction also showed that H-reflex amplitude recovered from the initial depression during stimulation at 10 and 20 Hz (Fig. 4). The 5-Hz stimulation did not evoke significant depression, and reflex amplitude did not change significantly throughout the stimulation. During 10-Hz stimulation, fast reflex recovery occurred, because *bin 1* (60% of H_1) was significantly larger than H_2 ($P < 0.05$), and slow recovery was also evident, because *bin 20* (86% of H_1) was significantly larger than *bin 1* ($P < 0.05$). Furthermore, *bin 20* and H_1 were not significantly different; thus complete recovery of reflex amplitude occurred by the end of the 10-Hz stimulation. During 20-Hz stimulation, fast recovery occurred, because *bin 1* (32% of H_1) was significantly larger than H_2 ($P < 0.05$), but slow recovery was not evident, because *bin 20* (36% of H_1) was not significantly different from *bin 1*. During the 20-Hz stimulation, reflex amplitude did not recover completely, because *bin 20* was significantly smaller than H_1 ($P < 0.001$).

Part 2: effect of background contraction. Figure 5 shows the mean amplitudes of M-waves and H-reflexes recorded during 10-Hz stimulation while participants were seated and holding different contraction levels in soleus. Qualitatively, M-waves showed initial depression for all contraction levels and no recovery, whereas H-reflexes showed more depression of reflex amplitude at lower levels of background contraction and similar recovery across most contraction levels. The five contraction levels, averaged across the group, were 0.4 ± 0.3, 1.8 ± 0.3, 5.3 ± 1.1, 9.8 ± 0.6, and 17.7 ± 1.7% MVC. There was a significant main effect of contraction level [$F_{(4,28)} = 459.9$, $P < 0.01$], and post hoc tests revealed that all contraction levels were significantly different from each other (data not shown). For M-waves, there were significant main effects of contraction level [$F_{(4,28)} = 4.1$, $P < 0.05$] and time [$F_{(3,21)} = 27.5$, $P < 0.01$]. The main effect of contraction level, collapsed across time, showed that M-wave amplitudes during the relaxed, 1%, and 5% MVC conditions were significantly smaller than during the 20% MVC condition ($P < 0.05$ for all comparisons). Post hoc analysis of the main effect of time showed that M_1 was significantly larger than the M-waves at the other three time points (M_2, *bin 1*, and *bin 20*; $P < 0.001$ for all comparisons) when the data were collapsed across all contraction levels. M-waves did not recover from this initial depression, because M_2, *bin 1*, and *bin 20* were not significantly different from each other.

For H-reflexes, there was a significant interaction between contraction level and time [$F_{(12,84)} = 12.3$, $P < 0.01$] (Fig. 6). The size of the first H-reflex in each stimulation train did not scale with contraction amplitude. However, the first H-reflex during the 1% MVC contraction was significantly smaller than the first H-reflexes during the 10% ($P < 0.05$) and 20% MVC contractions ($P < 0.01$). H-reflex depression occurred at all contraction levels, except 20% MVC. H_2 was significantly smaller than H_1 in the relaxed state (10% of H_1; $P < 0.01$) and at 1% (21% of H_1; $P < 0.01$), 5% (31% of H_1; $P < 0.01$), and 10% MVC (57% of H_1; $P < 0.01$). During the 20% MVC contraction, reflex amplitude did not change significantly throughout the stimulation, and thus these data are not discussed further. During the 10% MVC condition, H-reflexes were initially depressed and showed no recovery. The second H-reflex was not different from *bin 1* (fast recovery), and *bin 20* was not different from *bin 1* (slow recovery). There was significant recovery of reflex amplitude during the three lower contraction levels. In the relaxed condition, there was no significant fast recovery (H_2 was not different from *bin 1*), but there was slow recovery, because *bin 20* (40% of H_1) was significantly larger than *bin 1* ($P < 0.001$). For the 1% and 5% MVC contractions, fast, but not slow, recovery occurred. *Bin 1* (1% MVC, 54% of H_1; 5% MVC, 61% of H_1) was significantly

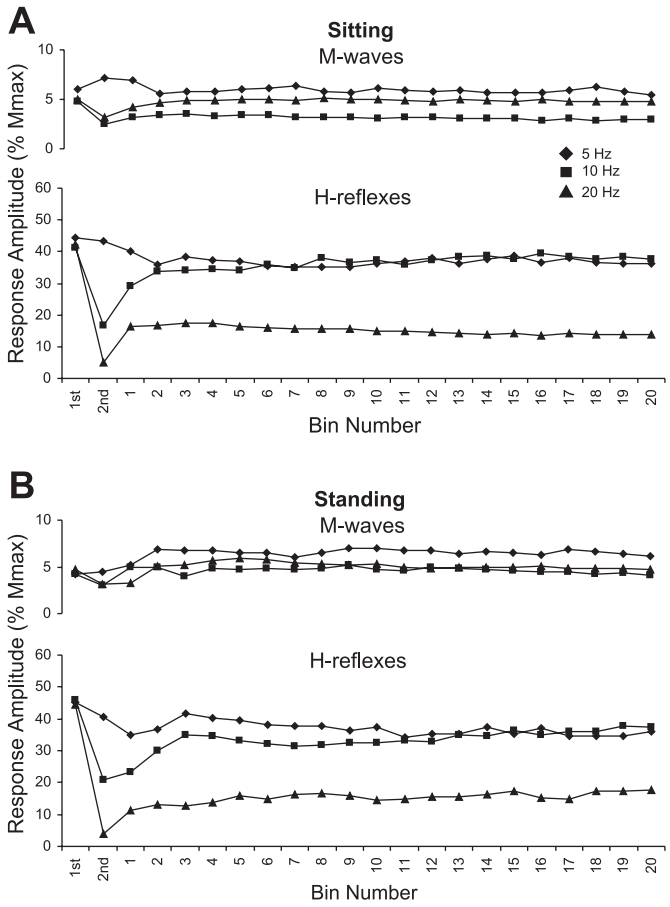

Fig. 3. Group average M-wave and H-reflex amplitudes during 10-s trains of stimulation delivered at 5, 10, and 20 Hz during sitting (*A*) and standing (*B*). The means of the first and second responses are shown, followed by the means of responses averaged over 0.5-s bins. Error bars have been omitted for clarity.

larger than H_2 ($P < 0.01$); however, *bin 20* and *bin 1* were not significantly different. Reflexes did not recover completely (i.e., back to H_1 amplitude) for any contraction amplitude.

As mentioned in the description of the single-participant data in Fig. 2, reflex amplitudes varied throughout the stimulation, and a marked recovery of H-reflex amplitude was observed in some participants by the third response. Although the variability in reflex amplitude often appeared to be random, in some participants a "pattern" emerged in which reflex

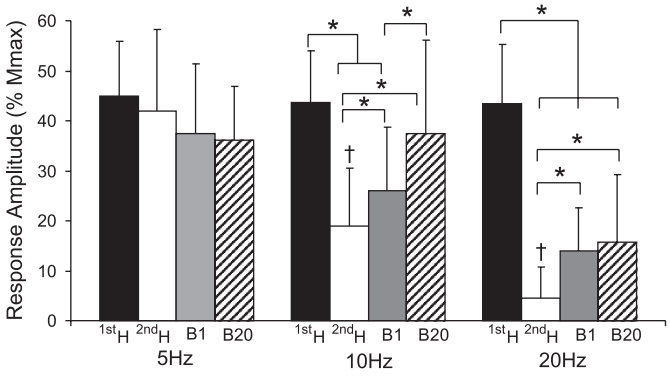

Fig. 4. Group data showing the significant interaction of stimulation frequency and time on H-reflex amplitudes. Data are collapsed over sitting and standing trials. Values are means (SD). Columns marked by crosses are significantly different from each other. *$P < 0.05$; †$P < 0.05$.

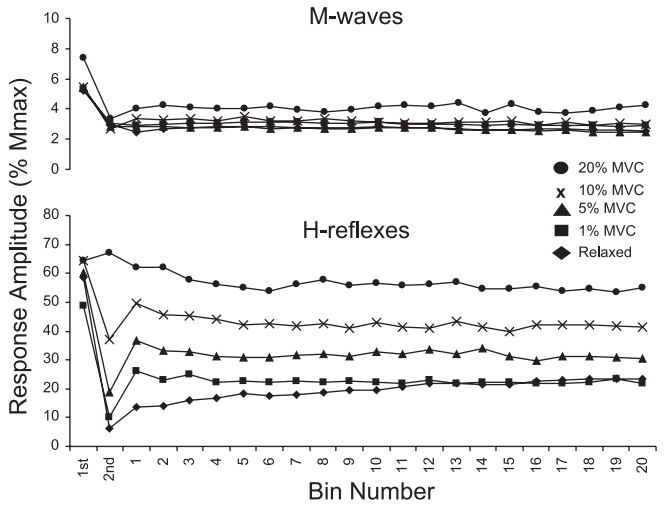

Fig. 5. Group average M-wave and H-reflex amplitudes during 10-s trains of 10-Hz stimulation during different levels of soleus background contraction. The means of the first and second responses are shown, followed by the means of responses averaged over 0.5-s bins. Error bars have been omitted for clarity.

amplitudes occasionally alternated between large and small (see Fig. 7) or between large, medium, and small (data not shown). Figure 7 provides an example of data from a participant in whom the third reflex was 19% larger than the first reflex, and a striking alternation of reflex amplitude, between ~30% M_{max} and 5% M_{max}, emerged while the participant was seated and holding a contraction of ~5% MVC in soleus. Although a detailed analysis of these apparent patterns in reflex expression was beyond the scope of the present study, we did quantify the fast recovery of reflex amplitude with a higher temporal resolution than permitted by the comparison of H_2 to *bin 1*. We compared the amplitudes of the first six H-reflexes across the group, and these results are shown in Fig. 8. Significant differences between H_1 and all other responses are identified by brackets in Fig. 8, but for clarity, other significant differences are not shown and are instead described below. There was a significant interaction between contraction level and time [$F_{(20,140)} = 9.5$, $P < 0.01$]. During the relaxed condition, H_1 was significantly larger than all other responses, and none of the other responses differed from each other. Thus reflexes were depressed and did not recover within the first six responses, and no alternation of reflex amplitude emerged. At

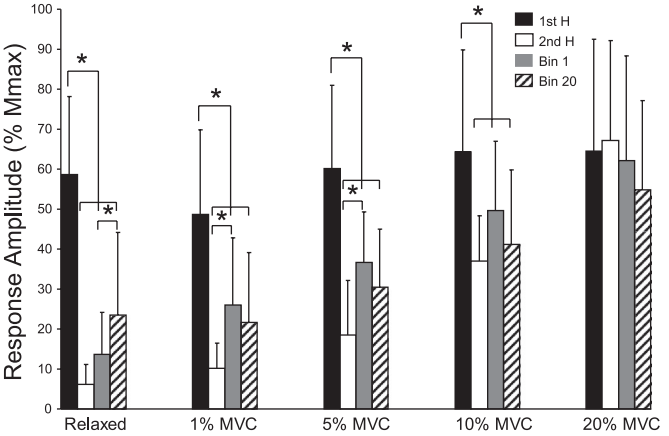

Fig. 6. Group data showing the significant interaction of background contraction and time on H-reflex amplitude. Values are means (SD). *$P < 0.05$.

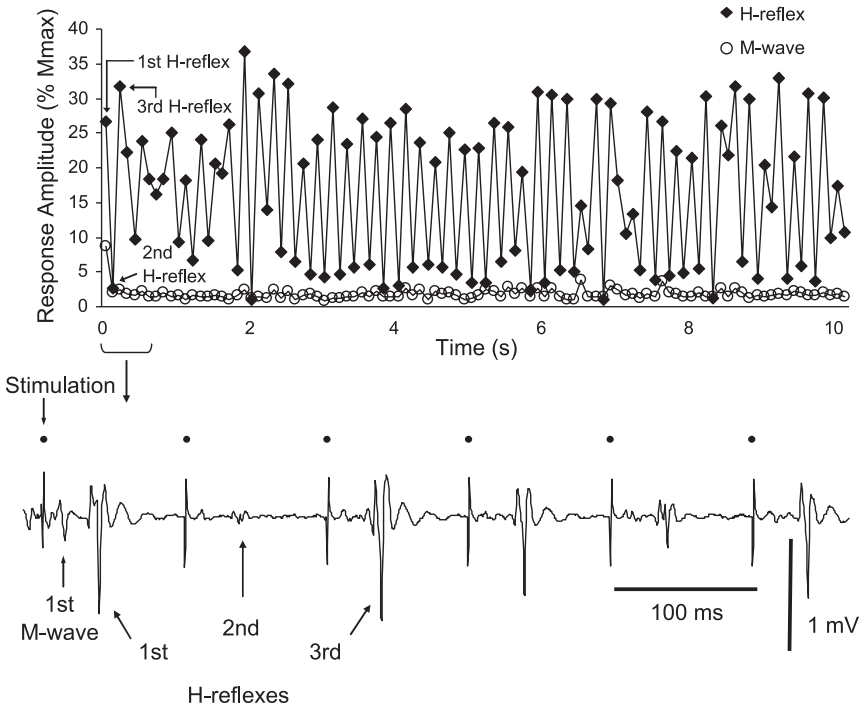

Fig. 7. Data from a single participant during a 10-s train of 10-Hz stimulation delivered when the participant was seated and holding a background soleus contraction of ~5% MVC. The data illustrate the strong alternation of reflex amplitudes that was observed in some participants. *Top*: M-waves and H-reflexes evoked by each stimulus pulse. *Bottom*: EMG responses to the first 6 stimulation pulses (filled circles denote stimulation artifacts).

1% MVC, H_1 was significantly different from all other responses, and H_2 was significantly different from H_3 and H_5, but not H_4 or H_6, indicating the emergence of alternating reflex amplitudes. At the 5% MVC level, complete recovery occurred by the third pulse, and a strong alternating pattern developed. Complete recovery was shown by the lack of difference between H_1 and H_3 amplitudes. The strong alternating pattern was highlighted by significant differences between all of the even numbered reflexes and odd numbered reflexes, not including the first response. Similarly, during the 10% MVC contraction, complete recovery of reflex amplitude occurred, because H_1 was not different from H_3 and H_5, and an alternation of reflex amplitude was evident, because H_2 was different from H_3 and H_5. Last, there were no significant differences between responses for the 20% MVC condition.

DISCUSSION

The results of the present study revealed that the depression of transmission along the H-reflex pathway, commonly known as PAD, was followed by significant recovery of reflex amplitude during 10-s trains of electrical stimulation. Stimulation frequency and the level of background contraction significantly influenced PAD&R, whereas changes in task (sitting or standing) had no effect on the depression or recovery of soleus H-reflexes. Although many studies have investigated PAD, this is the first study specifically designed to characterize the recovery of reflex amplitude during repetitive reflexive activation of motoneurons.

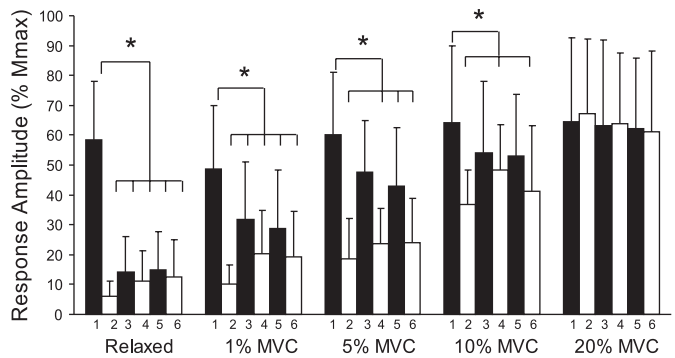

Fig. 8. Group data showing the significant interaction of background contraction and time (i.e., first 6 responses) on H-reflex amplitude. Values are means (SD). *$P < 0.05$, significant differences between H_1 and all other responses. For clarity, all other significant differences are not shown and are described in the text only.

In the current study, in addition to measuring the amplitude of the H-reflex evoked by each stimulus pulse, we also measured each corresponding M-wave as a measure of stimulation efficacy (Misiaszek 2003). Relatively few studies have measured M-waves when assessing PAD, and those that did reported no change in M-wave amplitude when H-reflexes were depressed (Floeter and Kohn 1997; Ishikawa et al. 1966; Jeon et al. 2007; McNulty et al. 2008; Trimble et al. 2000). In our study, although M-waves did not change during stimulation at 5 Hz, they were initially depressed and then remained stable during 10- and 20-Hz stimulation for all levels of background contraction. Plausible reasons why previous studies of PAD in humans have not reported a similar depression of M-waves are that in some cases the amplitude of the second M-wave was not reported (Trimble et al. 2000), stimulation frequencies above 5 Hz were not tested (Floeter and Kohn 1997; McNulty et al. 2008), or trials were excluded if the M-wave amplitude changed more than 2% between pulses (Jeon et al. 2007). A depression of M-wave amplitude during 20-Hz stimulation was recently reported when electrical stimulation was delivered using wide (500 and 1,000 μs), but not narrow (50 and 200 μs), pulse widths (Lagerquist and Collins 2010). This finding suggests that the M-wave depression stems from mechanisms related to the ability to repetitively activate motor axons beneath the stimulating electrodes, rather than reduced transmission across the neuromuscular junction or movement of the electrodes between pulses as a result of the muscle contraction.

A decreased ability to activate motor axons during repetitive stimulation raises the possibility that there also may have been a reduction in the ability to recruit sensory axons, which could have contributed to the H-reflex depression. However, since motor and sensory axons have different properties (Burke et al. 2001), it is difficult to translate changes in motor axon activation during repetitive stimulation to respective changes in sensory axon activation. Importantly, changes in M-wave amplitude between the first and second responses did not significantly account for changes in the H-reflex amplitude between the first and second responses across conditions, as indicated by analysis of covariance tests.

Effect of frequency on the depression and recovery of soleus H-reflexes. Our hypothesis about the relationship between PAD&R and stimulation frequency was supported by the finding that there was more depression and less recovery of soleus H-reflexes as stimulation frequency increased. While participants held a contraction of ~10% MVC, during 5-Hz stimulation there were no changes in reflex amplitude. During 10-Hz stimulation, there was significant depression of reflex amplitudes, followed by complete recovery by the end of the stimulation train. During 20-Hz stimulation, reflex amplitudes showed the greatest amount of depression, and this was followed by partial recovery of reflex amplitudes during the first 0.5 s of the stimulation train. These results are consistent with the well-known frequency dependence of PAD (Burke et al. 1989; Crone and Nielsen 1989; Ishikawa et al. 1966; Rothwell et al., 1986; Van Boxtel 1986), although our study is one of only a few to quantify PAD in humans at frequencies at or above 10 Hz (Goulart et al. 2000; Ishikawa et al. 1966; Jeon et al. 2007; Stein et al. 2007). Although other studies have found significant depression at frequencies less than 5 Hz, in most of these cases the participants were relaxed (Burke et al. 1989; Ishikawa et al. 1966; Rothwell et al. 1986; Van Boxtel 1986). The lack of PAD during 5-Hz stimulation in our study demonstrates the strong influence of contraction on the ability to measure PAD.

This relationship between PAD&R and stimulation frequency may be explained by several factors, including the ability to repetitively activate axons beneath the stimulating electrodes or changes in the presynaptic release of neurotransmitter or motoneuron excitability. Axonal excitability fluctuates when axons transmit trains of action potentials. At different intervals after an action potential, human sensory and motor axons express a relative refractory period (~3–4 ms), a supernormal period (~ 4–20 ms), and a subnormal period (~20–150 ms) (Burke et al. 2001; Kiernan et al. 1996). The subnormal period, in which axonal excitability is decreased, may have influenced the PAD&R we observed, because the interstimulus intervals for 10 and 20 Hz were 100 and 50 ms, respectively. Furthermore, during trains of stimulation, the effects of the subnormal period are summative, eventually leading to a plateau of axonal hyperpolarization (Bergmans and Michaux 1970; Bostock and Bergmans 1994). Such axonal hyperpolarization may have decreased the ability to activate axons repetitively, and thus the strength of the synaptic drive, more so during 20-Hz than 10-Hz stimulation.

The frequency dependence of PAD&R could also be related to several mechanisms that control neurotransmitter release. The mechanism most often associated with PAD is a decreased probability of neurotransmitter release from previously active Ia afferent terminals (Hirst et al. 1981; Hultborn et al. 1996; Kuno 1964). Activated Ia afferents can also evoke presynaptic inhibition on their own terminals (Eccles et al. 1962). This is not typically believed to contribute to PAD because the time course of presynaptic inhibition (up to 400 ms) is often shorter than the interstimulus intervals used in studies of PAD (Hultborn et al. 1996). In the present study, the interstimulus intervals were 200, 100, and 50 ms for 5, 10, and 20 Hz, respectively. Therefore, presynaptic inhibition could have been involved in the reflex depression observed in the current study. Regarding the recovery of reflex amplitudes, a possible presynaptic mechanism could be posttetanic potentiation. Posttetanic potentiation, which results in a prolonged increase in reflex amplitude following a period of repetitive afferent stimulation (Lloyd 1949), is thought to be caused by a lower probability of failure to release neurotransmitter from the presynaptic terminal, coinciding with a higher probably of multiquantal release (Hirst et al. 1981). In humans, posttetanic potentiation is typically evoked by delivering stimulation at frequencies greater than 100 Hz for seconds to minutes (Hagbarth 1962; Kitago et al. 2004; O'Leary et al. 1997; Van Boxtel 1986); although it has been shown during 3-s stimulation trains delivered at lower frequencies (10, 30 Hz) (Hughes et al. 1957).

At the level of the motoneuron, three additional mechanisms may influence the PAD&R that we observed. First, the afterhyperpolarization (AHP) can last up to ~100 ms for soleus motoneurons (Matthews 1996) and increases when motoneurons discharge repetitively (Gustafsson 1974; Ito and Oshima 1962; Wienecke et al. 2009). Thus, during the 10- and particularly the 20-Hz stimulation, the ability of the afferent volley to depolarize the motor pool was likely reduced by the AHP. Second, recurrent inhibition, induced by antidromic volleys in motor axons generated by the stimulation or through the reflexive activation of motoneurons, could have reduced the excitability of the motoneurons to repetitive input (Bussel and Pierrot-Deseilligny 1977; Eccles et al. 1954). The influence of antidromic recurrent inhibition on the current results was likely small due to the low stimulation intensity used, because M-waves were typically ~5% M_{max}. Finally, a gradual increase in the excitability of the motor pool during the stimulation may have contributed to the reflex recovery through the activation of persistent inward currents. Persistent inward currents enhance motoneuron excitability by amplifying synaptic input and helping to sustain motoneuron firing (Crone et al. 1988; Lee and Heckman 2000).

Effect of task on the depression and recovery of soleus H-reflexes. In support of our second hypothesis, PAD&R of reflex amplitudes were not influenced by task. We found no task-dependent differences in reflex depression or recovery between sitting and standing when background contraction and M-wave amplitudes were matched. Previous data available on the task dependence of PAD have been variable. Stein et al. (2007) found no depression of reflex amplitude when participants stood and held a soleus contraction of 15–20% MVC, but depression was evident when participants were seated and held similar levels of background contraction. Goulart et al. (2000) found no differences in PAD between sitting and standing when participants held similar background contractions between tasks. However, neither of these studies (Goulart et al. 2000; Stein et al. 2007) tested whether contraction levels were

significantly different between tasks, and M-waves were not measured. In another study, PAD was not different when participants were sitting or lying prone, over a range of background contraction levels (Trimble et al. 2000). Last, in a study in which M-waves amplitudes were controlled, PAD was not different when participants were lying prone with the soleus relaxed or standing while the tested leg was non-weight bearing (Jeon et al. 2007). It may be that differences in PAD previously attributed to task (Stein et al. 2007) may not be related to task per se; instead, if background contractions were larger during standing, the reduced PAD may have been more related to the well-known and strong effect of contraction on PAD (Burke et al. 1989; McNulty et al. 2008; Trimble et al. 2000).

Effect of background contraction on the depression and recovery of soleus H-reflexes. The hypothesis that H-reflex depression would scale inversely with increases in contraction level was supported by the current results. As the background contraction increased from rest, less depression of soleus H-reflexes was observed, and during the 20% MVC contraction, there was no depression. Our findings correspond with previous studies that found less depression with increasing levels of background contraction (McNulty et al. 2008; Stein et al. 2007; Trimble et al. 2000). This reduced ability to measure PAD may be due to a contraction amplitude-dependent decrease in synaptic efficacy caused by muscle spindle activation during the contraction (Hultborn and Nielsen 1998; Stein et al. 2007; Wood et al. 1996; see Introduction). On the basis of this idea, one might predict that the first reflex in each stimulus train would be progressively smaller as contraction amplitude increased. This was not the case, however, and the amplitudes of the first H-reflexes did not scale with contraction amplitude. It is likely that there was a trade-off between decreases in synaptic efficacy and increases in the excitability of the motor pool with increasing contraction amplitude. The relationship between motor unit size and PAD may also help explain the reduction in PAD as contraction amplitude increases. Small motor units exhibit more PAD than large motor units (Floeter and Kohn 1997; Van Boxtel 1986; cf. McNulty et al. 2008), and thus it may be more difficult to measure PAD at higher contraction amplitudes because as contraction amplitude increases, more of the small motor units are recruited by the voluntary contraction and do not respond to the afferent volley used to evoke the test reflex.

Rapid recovery of reflex transmission. Interestingly, the analysis of the first six reflexes in each stimulus train established that complete recovery of reflex amplitude was possible by the third reflex. This finding was surprising, because the mechanism most often attributed to PAD is a decreased probability of neurotransmitter release (Hirst et al. 1981; Hultborn et al. 1996; Kuno 1964). The current results are inconsistent with this classical mechanism, because it is unlikely that the probably of neurotransmitter release could vary to such a large extent between the second and third stimulation pulses during 10-Hz stimulation. If vesicle reuse or vesicle mobilization from the reserve pool (Zucker and Regehr 2002) were contributing to the complete recovery of reflex amplitude by the third stimulus pulse, one or both of these processes would need to work on a time course of ~200 ms. The time courses for both of these processes have been reported to range from hundreds of milliseconds to several seconds, depending on the preparation (Kavalali 2007; von Gersdorff and Matthews 1997; Zucker and Regehr 2002). It is evident that further work is required to verify the mechanisms behind the reflex depression, and depletion of neurotransmitter may not provide a full explanation. The mechanisms responsible for the alternation of reflex amplitude we observed are unclear; however, possibilities include changes in axonal excitability or the duration of the AHP relative to the timing of each stimulation pulse.

Summary. We studied PAD&R of reflex transmission by delivering trains of stimulation at physiologically relevant frequencies during functionally relevant tasks and contraction levels. Transmission along the H-reflex pathway was strongly influenced by stimulation frequency and background contraction amplitude. On the contrary, there were no task-dependent differences in PAD&R of reflex amplitudes between sitting and standing. After the initial PAD, reflex amplitude recovered completely by the end of the 10-Hz stimulation, which emphasizes that transmission along the H-reflex pathway does not remain depressed after the first pulse during repetitive stimulation. In addition, a complete recovery of reflex amplitude could occur by the third pulse within a stimulation train, a finding that is not consistent with classical ideas regarding the mechanism of PAD. Our results support the idea that there is an ongoing interplay between depression and facilitation of reflex transmission during trains of afferent input (Lloyd 1949, 1958). In the present study we have shown that this balance between depression and facilitation depends strongly on the frequency of the afferent input and the magnitude of the background contraction but is relatively insensitive to changes in task.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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