Postactivation Depression of the Soleus H Reflex Measured Using Threshold Tracking

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McNulty PA, Jankelowitz SK, Wiendels TM, Burke D. Postactivation depression of the soleus H reflex measured using threshold tracking. J Neurophysiol 100: 3275–3284, 2008. First published October 15, 2008; doi:10.1152/jn.90435.2008. The interpretation of changes in the soleus H reflex is problematic in the face of reflex gain changes, a nonlinear input/output relationship for the motoneuron pool, and a nonhomogeneous response of different motoneurons to afferent inputs. By altering the stimulus intensity to maintain a constant reflex output, threshold tracking allows a relatively constant population of α-motoneurons to be studied. This approach was used to examine postactivation (“homosynaptic”) depression of the H reflex (HD) in 23 neurologically healthy subjects. The H reflex was elicited by tibial nerve stimulation at 0.05, 0.1, 0.3, 1, and 2 Hz at rest and during voluntary plantar flexion at 2.5, 5, and 10% of maximum. A computerized threshold tracking procedure was used to set the current needed to generate a target H reflex 10% of \( M_{\text{max}} \). The current needed to produce the target reflex increased with stimulus rate but not significantly beyond 1 Hz. In three subjects, the current needed to produce H reflexes of 5, 10, and 20% \( M_{\text{max}} \) at 0.3, 1, and 2 Hz increased with rate and with the size of the test H reflex. HD was significantly reduced during voluntary contractions. Using threshold tracking, HD was maximal at lower frequencies than previously emphasized, probably because HD is greater the larger the test H reflex. This would reinforce the greater sensitivity of small motoneurons to reflex inputs.

INTRODUCTION

The H reflex is a relatively simple, largely monosynaptic, spinal reflex, dependent on muscle spindle group Ia afferent excitation of spinal motoneurons and has therefore been considered the electrical analog of the spinal stretch reflex. Changes in the reflex with the rate of stimulation (Hoehler et al. 1981), level of voluntary contraction (Capaday and Stein 1987), posture (Goulart et al. 2000), arousal (Brunia 1971; Bathien and Morin 1972), task (Schneider et al. 2000), muscle length (Garland et al. 1994), and time (Crone et al. 1999) indicate that the underlying reflex mechanisms are more complex than originally supposed. It has been argued that the H reflex is not exclusively monosynaptic (Burke et al. 1984) and, more recently, evidence has been presented that reflex size is, indeed, limited by group Ib afferents in the test volley (Marchand-Pauvert et al. 2002).

With increasing stimulation rates within the low-frequency range, the amplitude of the group Ia monosynaptic response is reduced (Eccles and Rall 1951; Lloyd and Wilson 1957) because of attenuation of the excitatory postsynaptic potential (EPSP) (Curtis and Eccles 1960; Lüscher et al. 1983; Lev-Tov and Pinco 1992). Maximal depression occurs with stimulus rates of 3–5 Hz. This postactivation depression is also termed “homosynaptic” depression (Bewsick and Evanson 1957; Capek and Esplin 1977; Eccles and Rall 1951) and, in this paper, that term and its abbreviation, HD, will be preferred, in part to avoid potential confusion about mechanisms created by the abbreviation PAD. However, repetitive activation of group Ia afferents across both the low- and high-frequency ranges has complex effects on the EPSP produced in target motoneurons. Such changes are dependent on discharge rate and the target motoneuron and may involve branch-point failure of transmission in axon terminals in addition to any effects on transmitter availability, again changes that are strictly homosynaptic (Collins et al. 1988; Edwards et al. 1976a,b; Honig et al. 1983; Lev-Tov and Pinco 1992; Lüscher et al. 1983). For frequencies ≥5 Hz, the data of Lev-Tov and Pinco (1992) suggest that the frequency-dependent depression of the ESPSP cannot be attributed to failure of the action potential to invade Ia terminals.

The H reflex undergoes less HD when the test motoneuron pool is voluntarily active (Burke et al. 1989; Rothwell et al. 1986; Stein et al. 2007). It has been suggested that HD is not functionally important (Stein et al. 2007). However, on the one hand, short-term skilled training depresses HD of the H reflex for ≥24 h (Meunier et al. 2007), and this mechanism could underlie the smaller H reflex after long-term skilled training in ballet dancers compared with controls (Nielsen et al. 1993). On the other hand, for reflexes in the upper and lower limbs, HD is consistently less in patients with spasticity caused by cerebral or spinal lesions (Aymard et al. 2000; Gray et al. 2008; Hultborn and Nielsen 1998; Ishikawa et al. 1966; Lamy et al. 2005; Nielsen et al. 1995; Schindler-Ivens and Shields 2000; see Pierrot-Deseilligny and Burke 2005).

When the H reflex is used to study spinal mechanisms, problems can occur if afferent inputs are not distributed homogeneously to motoneurons in the target pool, if the input-output relationship of the pool is not linear, or if there is a change in recruitment gain for the motoneuron pool (Crone et al. 1990; Garnett and Stephens 1981; Kernels and Hultborn 1990; Nielsen and Kagamihara 1993; see Pierrot-Deseilligny and Burke 2005). These issues are avoided in an approach developed by Shindo et al. (1994), who studied the H reflex in single motor units varying the stimulus intensity until the single unit could be activated with a 50% firing probability at rest and during experimental maneuvers. The technique re-
quires the isolation of a single motor unit and is biased toward low-threshold motoneurons. In studies of the compound H reflex, the second issue is now usually addressed by using an unconditioned test reflex that is 10–20% of the maximal M wave (M_max), within the linear range of the input-output relationship (Crone et al. 1990). In this study, we adopted a third approach that combines the advantages of the other two approaches: threshold tracking of a compound H reflex potential, altering the input by adjusting the stimulus current needed to generate a response of predetermined amplitude (Lin et al. 2002). This effectively clamps the output so that the reflex response involves a constant population of α-motoneurons from a larger pool. As a result, variations in the stimulus current needed to produce the test reflex reflect changes in the net synaptic drive onto the motoneuron pool, data from which insights into the compound EPSP can be derived. With this technique, HD was found to reach a plateau at relatively low stimulus rates (~1 Hz) and to increase with the size of the test H reflex, unexpected findings given previous studies using more conventional reflex recording techniques.

**METHODS**

**Subjects**

Data were collected in 27 experiments in two series: one at rest and one with voluntary contractions. Twenty-three neurologically healthy subjects participated (11 women and 12 men; mean age, 29 yr; age range, 19–52 yr). Four subjects, including one of the authors, participated in more than one experiment. The test side was randomly chosen, with 16 studies on the right leg and 11 on the left. In four experiments, studies on both legs were undertaken to enable comparisons between sides for the same subject. All subjects gave signed, informed consent to the studies that had been approved by the Human Research Ethics Committee, University of Sydney.

Subjects sat with the test leg firmly fixed to a force transducer via Velcro straps around the ankle and instep. To reduce muscle stretch and minimize potential contributions to the H reflex by the gastrocnemius muscle, the knee was flexed ~30° from full extension. Because the H reflex attenuates with ankle dorsiflexion (Delwaide 1973; Hoehler et al. 1981), the ankle was plantar flexed ~30° from a right angle (Fig. 1A). To minimize changes in alertness, subjects were seated in a comfortable chair and were able to relax fully. Distractions in the laboratory were minimized throughout the recording period.

Soleus EMG was recorded using disposable 10-mm Ag/AgCl electrodes 4 cm apart positioned immediately below the division of the two heads of the gastrocnemius in the midline over the soleus muscle. EMG was amplified (~500–1000), filtered (1–1000 Hz), and digitized at 10 kHz. Isometric plantarflexion force was monitored using a 1-kN load cell (XTran, Applied Measurements, Victoria, Australia) filtered from DC to 20 Hz and sampled at 10 kHz.

**Stimulation**

A computer-controlled, isolated constant-current source under control of the QTRAC threshold tracking program (Prof. Hugh Bostock, Institute of Neurology, London, UK) was used to stimulate the tibial nerve. The nerve was stimulated in the popliteal fossa near the midline of the knee flexion crease using a 4-mm Ag/AgCl surface probe to deliver square-wave depolarizing pulses 1 ms wide. The anode was positioned over the patellar tendon. Once the optimal stimulation site was located, the cathode was firmly secured in place using an elastic belt. When the test stimulus is altered to track an H reflex of constant size, an increase in central excitability will produce a decrease in the current necessary to generate the test reflex. This contrasts with the increase in the H reflex that occurs with a fixed test stimulus (Fig. 2).

Each study began with a stimulus-response curve (Fig. 3A) to define the current needed to generate a maximal muscle response (M_max) and an M wave that was 10% of M_max (10% M_max). A cycle of stimuli with equal interstimulus intervals produced 1) M_max using a fixed stimulus that was 10% greater than needed for M_max, 2) an M wave with an amplitude 10% M_max, and 3) an H reflex to a tracking stimulus, with a target size 10 ± 0.5% of M_max. This H reflex amplitude ensured that the response remained on the ascending limb of the H reflex recruitment curve even with sizeable increases in stimulus current. As can be appreciated from Fig. 3, if reflex responses fell on the descending limb of the recruitment curve, a reflex response that was smaller than the 10% target would lead to an increase in stimulus current, and this would produce an even smaller reflex response, prompting a further increase in stimulus intensity. The stimulus current could be reset manually if this occurred. In three subjects, H reflexes 20% of M_max could be studied because they had large M_max/M_max ratios.

M_max was monitored throughout each experiment to ensure that changes in the H reflex were not caused by fatigue or movement of electrodes. However, referencing the target size to an updated M_max helped to ensure that this would not have created issues, had it occurred. The intensity for each stimulus in the three-stimulus sequence was either constant, set to produce a potential of predetermined amplitude under control conditions (e.g., M_max and submaximal M wave 10% M_max), or adjusted by the computer dependent on the amplitude of the previous reflex response (H reflex 10% M_max). The current for the tracked response changed from trial to trial, increasing or decreasing in proportion to the tracking error (i.e., the deviation of the previous response from the target). This stimulus sequence is shown in Fig. 1B. Stimuli recurred in repeating trains at 0.1, 0.3, 1, and 2 Hz. For four experiments, 0.05 Hz was added and, in a single experiment, repetition rates of 0.025, 3, and 4 Hz were also studied. The order of stimulus frequencies was randomly varied for the two slow and two fast frequencies.

For each stimulus repetition rate, the protocol had four phases based on the stimulus response curve: 1) three stimuli: a supramaximal stimulus for M_max, a submaximal stimulus tracking an M wave that was 10% M_max, and a submaximal stimulus tracking an H reflex that was 10% M_max; 2) three stimuli: fixed stimuli for M_max and also for the submaximal M wave and a variable stimulus tracking the target H reflex; 3) two stimuli: a fixed stimulus for the submaximal M wave and a variable stimulus tracking the target H reflex; 3) two stimuli: a fixed stimulus for the submaximal M wave, and a variable stimulus tracking the target H reflex (see Fig. 1B). Thus phase 1 was used to determine the currents needed to generate H and M waves that were 10% of M_max at 0.3 Hz. Phase 2 was a test phase with the computer cycling through the three different stimuli in turn, whereas phase 3 was a test phase with the supramaximal stimulus omitted. Phase 4 was used to ensure that M_max had not changed. The duration of phase 1 depended on the number of cycles taken for the M wave and the H reflex to track to 10% M_max phases 2 and 3 contained.

**Fig. 1.** Protocols for the experiments. A: experimental set up. B: stimuli were delivered on 3 separate channels. Filled squares, supramaximal stimulus to generate a maximal M wave, set to 110% of that needed for M_max, filled circles, M wave tracked to 10% M_max and fixed at that intensity; open circles, H reflex tracked to 10 ± 0.5% M_max. Top: timing and amplitude of stimuli delivered during 1 experiment; the tracking error boundaries for the H reflex channel are indicated by the dashed lines. The open rectangle on the far right of the H reflex data represents the mean current (±SE) used in this recording. Bottom: the 4 phases of the stimulus protocol separated in time to highlight changes the stimulus in each phase. C: the more conventional method of studying the H reflex uses a stimulus of constant intensity, and this results in an H reflex of variable amplitude even at rest in the absence of conditioning. The rectangular bar at the far right indicates means ± SE (n = 35).

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A

[Diagram showing a person sitting on a chair with a force transducer and a screen display for force & target. The screen displays waveforms for soleus EMG and tibial nerve stimulation.]

B

<table>
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<th>current intensity</th>
<th>supramax</th>
<th>110% Mmax</th>
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<td>M wave</td>
<td>tracking</td>
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<td>H reflex</td>
<td>tracking</td>
<td>10% Mmax</td>
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C

constant current intensity - input
measured EMG response - output

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15 cycles, and phase 4 contained 3 cycles. The stimulus current for the test H reflex was always less than that for the 10% M wave (see Fig. 5A), so that in each phase of the protocol the effective stimulus rate for the afferents producing the H reflex was the overall repetition rate and not the recurrence rate of the H reflex stimulus. The test period was phase 3, by which time the degree of HD would have been at steady state for that stimulus rate.

Based on results obtained in the preceding experiments, the number of cycles was reduced for the voluntary contraction studies to minimize the potential for fatigue at these low contraction levels (see Søgaard et al. 2006). Accordingly, phase 2 was reduced to three cycles. For each stimulus frequency, the 10% M wave was tracked during phase 1 and set for the remainder of the protocol. The H reflex was tracked throughout. For the voluntary contraction series, stimulus

**FIG. 2.** Contrasting behavior of the H reflex in fixed stimulus and threshold tracking protocols during a voluntary contraction of soleus. The top trace shows the fixed stimulus current that evoked H reflexes of amplitude shown in the 2nd trace. Note that voluntary contraction (indicated by the EMG in the lowest trace) increased the reflex response to a fixed stimulus. With threshold tracking (3rd and 4th traces), the stimulus was adjusted to produce an H reflex of constant amplitude, and this was effective except at the onset and offset of the contraction (4th trace). The stimulus current necessary to produce the H reflex decreased during the contraction and returned to control levels when the contraction ceased. Note the delay taken before the tracking stimulus reaches the appropriate level at both the onset and offset of the contraction.

**FIG. 3.** Stimulus/response curve from a single experiment. A: the top trace shows the steady increase in the stimulus current from below motor threshold to M_max, producing the H reflexes and M waves shown in the bottom traces in A. Note that the current needed to produce an H reflex that was 10% M_max was less than needed for an M wave of the same size. B: 3 traces of raw data obtained at the letters indicated in A: a, an H reflex but no M wave; b, a larger H reflex and an M wave; c, a larger M wave and smaller H reflex. The 15-ms measurement windows are shown below the M waves and H reflexes.
frequencies were restricted to 0.1, 0.3, and 1 Hz, and the protocol was repeated four times: at rest and during voluntary plantar-flexion at 2.5, 5, and 10% of a maximal voluntary contraction (MVC), performed in this order to minimize fatigue, with several minutes of rest between each repetition. Subjects were given visual feedback of the generated force and the target level. They were asked to match their force to the target level and hold it there until instructed to relax between changes in frequency. All subjects were able to sustain the voluntary contractions for the required time. To establish contraction levels, subjects performed three brief MVCs lasting 2–3 s in the test position. They were asked to limit activation to the calf muscles and were given verbal encouragement and visual feedback. In three experiments, MVCs were repeated after each contraction level to assess fatigue. To accommodate the greater variability during sustained contractions, the allowable tracking error for the H reflex was increased to ±10% (i.e., ±1% \( M_{\text{max}} \)) for these studies.

Data analysis

The amplitude of the M and H responses were measured peak-to-peak. The threshold for the H reflex was measured as the current needed to produce an H reflex that was 10% of \( M_{\text{max}} \). To enable comparisons across subjects, the threshold for the H reflex was normalized to the current needed to elicit an M wave 50% of \( M_{\text{max}} \), based on the stimulus-response curve. This was more accurate than using the current for \( M_{\text{max}} \) because the reference current was then on the steeply rising portion of the stimulus-response curve rather than where it was beginning to plateau. The responses during each phase for each stimulus intensity were averaged. The amplitudes of the M wave and H reflex and the normalized H reflex threshold were expressed as a percentage of the average response at 0.1 Hz. Normally distributed data were analyzed using one-way ANOVA and paired \( t \)-test. For nonparametric data, a Kruskal-Wallis rank sum ANOVA with post hoc Dunn’s analysis or Mann Whitney rank sum test were used. Data are presented as means ± SE, and differences were considered significant when \( P < 0.05 \).

**RESULTS**

**H reflex threshold current at rest**

The current needed to generate an H reflex at rest with an amplitude of 10% \( M_{\text{max}} \) was significantly different for different rates of stimulation (\( P < 0.001 \); Fig. 4A). The current needed for the test reflex was normalized to that at 0.1 Hz and was 15.0 ± 0.53% greater at 1 Hz than at 0.1 Hz (\( P = 0.009 \)) and 5.4 ± 0.29% greater at 0.3 Hz than at 0.1 Hz (\( P = 0.016 \)). There were no significant differences in the threshold currents for 1 and 2 Hz or for 0.05 and 0.1 Hz. However, the latter result is based on only four recordings at the slower rate (0.05 Hz). These results were repeated in a single subject with additional stimulation rates of 0.025, 3.0, and 4.0 Hz (Fig. 4B). In this subject, the curve was shifted to the left compared with the population response in Fig. 4A. The overall dependence on stimulation rate was significant (\( P < 0.001 \)), but the increase in current between 0.3 and 1.0 Hz was only 2.0 ± 0.15% (\( P < 0.001 \)), whereas between 0.3 and 0.1 Hz, it was 4.8 ± 0.22% (\( P < 0.001 \)), the same as the population response. When measured as an increase in threshold current for the target H reflex, HD was near maximal at 0.3 Hz and was on the steeply rising portion of the curve at 0.1 Hz. It is relevant that, with rates <0.1 Hz, some subjects are not capable of maintaining a constant attention level, whereas stimulation rates >1 Hz can be difficult for threshold tracking because reflex fluctuations can cause threshold increases that place the response on the descending limb of the (attenuated) H reflex curve.

The data in Fig. 4, A and B, are for H reflexes set to 10% \( M_{\text{max}} \). To determine whether HD differed for motoneurons of different threshold, the H reflex was measured for reflexes of different size (5, 10, 15, and 20% \( M_{\text{max}} \)) at 0.3, 1.0, and 2.0 Hz (Fig. 4C) in three subjects at rest. Larger H reflexes could not be studied because of the risk that the reflex responses would...
be near $H_{\text{max}}$ (see METHODS). In these studies, the data for each H reflex size were normalized to the threshold for the target reflex at 0.3 Hz. HD was greater for the larger H reflex (Fig. 4, C and D). There was a significant two-way interaction between stimulus frequency and the size of the test reflex ($P < 0.001$). Significant differences were found for the size of the reflex and for the rate of stimulation ($P < 0.001$ for both). Post hoc analysis showed significant differences between all reflex sizes except between 5 and 10%. There was no difference for data recorded at 0.3 and 1 Hz between 15 and 20% $M_{\text{max}}$. For the individual subject, all points were significantly different ($P < 0.001$) except those at 0.3 Hz and between 1 and 2 Hz at 10% $M_{\text{max}}$ (Fig. 4D).

Previous data have suggested that small H reflexes (or monosynaptic reflexes) undergo greater attenuation with rate (Cook 1968; Field-Fote et al. 2006; Floeter and Kohn 1997; Lloyd and Wilson 1957; Rossi-Durand et al. 1999; van Boxtel 1986). Contrary to this, these data indicate that, when HD is measured as the change in threshold current for a constant H reflex size, the magnitude of HD increases the larger the test H reflex, i.e., when more motoneurons are recruited into the test reflex. For a target H reflex 10% $M_{\text{max}}$, HD was near maximal at 1 Hz, ~6% more current being needed than at 0.3 Hz. When the target H reflex was 20% $M_{\text{max}}$, 9% more current was needed at 1 Hz than at 0.3 Hz, and even more current was needed at 2 Hz (10.5%; $P < 0.001$). In other words, HD for small reflexes was less and tended to reach a plateau with increasing rate earlier than HD for large reflexes.

Comparison of HD measured as a decrease in reflex amplitude and an increase in reflex threshold

In four experiments, we compared HD measured by tracking the threshold current for a constant reflex size with HD measured by changes in the peak-to-peak amplitude of the H reflex to a constant stimulus intensity. The test H reflex was 10% $M_{\text{max}}$ at 0.1 Hz (fixed current). Changes in HD were assessed at 0.1, 0.3, 1.0, and 2.0 Hz. Using control reflexes of the same size, there was no significant difference in the rate of development of HD with the two methods of measurement (Fig. 5). There was a significant two-way interaction (rate $\times$ method; $P < 0.001$; Fig. 5A). Significant differences were found for both the method and for stimulation rate ($P < 0.001$). Post hoc pair-wise comparisons found significant differences for the tracked current only between 0.1 and 2 Hz (Fig. 5A). For the fixed current, significant differences were found for all comparisons except 1 Hz with 0.3 and 2 Hz (Fig. 5B). When expressed as a percentage of the control level, the extent of the HD measured using threshold tracking was significantly smaller than that seen using a fixed current.

When the current was fixed, the mean reflex amplitude variability [expressed as the coefficient of variability (CoV)] decreased with increasing stimulation rate, from 0.086 at 2 Hz to 0.033 at 0.1 Hz (Fig. 5B). The variability of the current used in threshold tracking showed no such trend (Fig. 5B). The mean CoV for the fixed current was 0.065 and for the tracked current was 0.003, a difference that was significant ($P < 0.001$, paired t-test). The 20-fold variability with threshold tracking indicates greater sensitivity for threshold tracking despite the smaller percentage change. The decrease in reflex amplitude (fixed current) was plotted against the increase in threshold (tracked current) for each stimulus rate for each subject (Fig. 5D). There was a biphasic relationship with large changes in the reflex amplitude when the changes in tracked threshold were small, but when reflex amplitude was severely attenuated, there were large changes in threshold.

At rest, HD was minimal at the stimulus rate of 0.05 Hz (Fig. 4A), and this was confirmed in one subject with a wider stimulus range (Fig. 4B). At this rate, the ratio of the threshold for a 10% H reflex and the threshold for the 10% $M$ wave was 0.6 (Fig. 6A). This matches the threshold for the H reflex of single motor units in both quadriceps femoris and tibialis
and weak (2.5%) MVCs. The greatest decrease in threshold occurs between rest and voluntary contraction, a small rate effect can still be seen at the normalized threshold for 0.1 Hz at rest. Although HD has been largely attenuated by the voluntary contraction, a small rate effect can still be seen at all contraction levels. The greatest decrease in threshold occurs between rest and weak (2.5%) MVCs.

The threshold current for the H reflex during constant-force voluntary contractions was significantly less than that needed for the maximal H reflex between 1 and 2 Hz, with values of 0.79 ± 0.065 and 0.81 ± 0.067, respectively. Accordingly, at all tested stimulus frequencies, the threshold for the target H reflex was below that for the 10% Mmax, at 5 stimulus frequencies. Data from 9 subjects (except at 0.05 Hz where n = 4). There is no significant change in the ratio after 1 Hz. The ratio is maximal at 0.05 Hz (0.6). The change in H reflex threshold with constant force voluntary contractions of different size: 2.5% maximal voluntary contraction (MVC) solid bars, 5% empty bars, 10% hatched bars. The dashed line indicates the normalized threshold for 0.1 Hz at rest. Although HD has been largely attenuated by the voluntary contraction, a small rate effect can still be seen at all contraction levels. The greatest decrease in threshold occurs between rest and weak (2.5%) MVCs.

antierior (0.6 × motor threshold; see Meunier et al. 1990) and supports the view that threshold tracking of the H reflex involves changes in the activation of soleus Ia afferents. At 10% Mmax, there was no change in the “H:M threshold ratio” between 1 and 2 Hz, with values of 0.79 ± 0.065 and 0.81 ± 0.067, respectively. Accordingly, at all tested stimulus frequencies, the threshold for the target H reflex was below that for the 10% M wave, as might be expected given the differences in the strength-duration properties of Ia afferents and α-motor axons (Lin et al. 2002).

**H reflex threshold during voluntary contractions**

The threshold current for the H reflex during constant-force voluntary contractions was significantly less than that needed at 0.1 Hz at rest (P < 0.001; Fig. 6A). There was no interaction between the strength of the voluntary contraction and the frequency of stimulation, but there was a significant effect of the strength of the voluntary contraction (P < 0.001). This was greatest on going from rest to contraction rather than between contraction levels (Fig. 6B). The changes in threshold current between contraction levels were not significant, but there were trends for a lesser current with a stronger contraction and for a greater current with a higher stimulus rate, particularly when the contraction level was 2.5% MVC.

**Amplitude of the M wave and H reflex**

The amplitude of the Mmax did not change between rest and voluntary contraction, with different levels of voluntary contraction or with different rates of stimulation. There was also no change in the amplitude of the 10% Mmax in any condition. This suggests that the observed differences in HD were not complicated by electrode movement, changes in leg position, greater synchrony within the activated motor units, or a change in the excitability of motor axons. The amplitude of the H reflex tracked to be 10% Mmax did not change across conditions even though the threshold current needed to produce the 10% test H reflex changed across conditions. In four subjects, the protocol was performed on both legs. There were no significant differences in the normalized threshold or amplitude of the maximal H reflex between sides. Both right and left recordings were made on the same day for each subject, with the leg to be studied first randomly varied to minimize transfer or learning effects. In three experiments, the amplitude of the MVC was measured after the 10% MVC. There was no significant difference in the pre- and post-trial MVCs, either for the pooled data or for individual subjects. These results suggest that fatigue did not contribute to the patterns of reflex change seen in this study.

**DISCUSSION**

This study explored the phenomenon of HD using computerized threshold tracking to restrict the reflex response to a fixed population of motoneurons. As discussed later in the discussion, threshold tracking avoids the “pool problems” that can confound the interpretation of H reflex studies. The differences in HD reported here were measured as changes in the stimulus current necessary to produce the target H reflex. This would result in a change in the number of Ia afferents activated by the test stimulus and thereby to a change in the input to the motoneuron pool. With this technique, HD seems to be maximal at lower frequencies than has been reported in previous studies that measured the change in reflex size to a fixed stimulus. This is likely to be caused by differential susceptibility of motoneurons at different recruitment levels to changes in their Ia afferent input. Contrary to previously reported findings, HD is greater and not smaller for larger reflexes when measured using the present technique.

**Methodological issues**

In the H reflex, motoneuron discharge depends on a compound group I EPSP, with the true extent of Ia excitation partly offset by Ib inhibition. The group Ib component of the afferent volley changes with stimulus strength, much as the Ia component, and this is potentially a confounding factor in this study. When subjects were at rest, it is likely that the relative Ia and Ib contributions to the compound group I EPSP were similar over the limited stimulus ranges used in this study. However, during tonic voluntary contractions, the excitability of nonreciprocal group I inhibitory interneurons (Ib inhibitory interneur-
rons) projecting to the active motoneurons is decreased (Fournier et al. 1983), and this changes the balance between Ia excitation and Ib inhibition in the compound group I EPSP (Burke et al. 1984; Marchand-Pauvert et al. 2002). This change would presumably contribute to the contraction-associated potentiation of reflex excitability. In addition, it is possible that suppression of a Ib limitation on the group I EPSP is a factor in the contraction-associated decrease in HD. The motoneurons most susceptible to disynaptic inhibition are the higher-threshold motoneurons contributing to the reflex discharge. As suggested in Fig. 4 and argued below, HD increases with the size of the test H reflex, presumably because transmitter depletion is more prominent on Ia terminals on higher-threshold motoneurons.

Direct comparison of the two methods for measuring HD for H reflexes initially 10% of M_max in the same subjects did not show any substantial difference in the rate sensitivity of the two techniques. This was contrary to our initial hypothesis. However, the comparison did confirm a much lower variability with threshold tracking, and this suggests that threshold tracking might provide a more reliable measure of the phenomenon. A major disadvantage of measuring the change in amplitude of the H reflex to a constant stimulus intensity is that, of necessity, the test reflex will change during the stimulus train. Given that the extent of HD varies with reflex size, this introduces a further variable. The threshold tracking technique avoids this potentially confounding issue.

Advantages and limitations of threshold tracking of H reflexes

One of the principle uses for the H reflex is to examine the excitability of spinal cord circuitry. Conventional H reflex methods use a fixed stimulus current intensity that will activate a constant population of Ia (and Ib) afferents. The variability in the resulting H reflex (Funase and Miles 1999) results from changes in the population of α-motoneurons recruited into the reflex from trial to trial, because of intrinsic motoneuron factors and/or changes in their input, both excitatory and inhibitory. The combination of these factors may impoverish the information that can be inferred from such reflex studies. On the other hand, threshold tracking clamps reflex output to a relatively constant population of motoneurons, producing a stable reflex amplitude (Chan et al. 2002). The recruitment of a stable population of motoneurons avoids pool problems inherent in conventional reflex studies. It minimizes the possibility that nonlinearity of the input-output relationship or nonhomogeneous distribution of afferent inputs would be interpreted as a change in reflex transmission, and changes in reflex gain can be detected if the threshold tracking involves target reflexes of different size.

Transmission through most spinal reflex circuits is maximal at the onset of a voluntary contraction (see Pierrot-Deseilligny and Burke 2005), and a major limitation of threshold tracking of the H reflex is the time taken for the current to track to the appropriate level, as seen in Figs. 1B and 2. When there is an abrupt large change in reflex excitability, threshold tracking can underestimate the extent of that change, but this is not an issue with steady-state measurements, as used in this study. On the other hand, the changes that occur in the H reflex with a fixed stimulus current can be manifest fully in the next trial. Which method of measuring changes in reflex excitability is preferable will depend on the question being asked and whether pool problems are a critical issue. These data provide further confirmation that H reflexes of different size may respond differently to changes in the same input, and this cautions against comparing reflexes of different sizes (see Crone et al. 1990).

Threshold tracking works best when the H_max-M_max ratio is high so that the amplitude of the test H reflex does not approach H_max. If the target H reflex is close to H_max, a decrease in central excitability will produce a small increase in stimulus current, and this can move the test reflex onto the descending limb of the H reflex recruitment curve. Thus this method of studying the reflex may be less useful in older subjects who commonly have a low H:M ratio (Kido et al. 2004), and it is inappropriate for studying the reflex responses of relatively high-threshold motoneurons.

Threshold tracking relies on varying the stimulus intensity to keep a reflex discharge constant, and this necessarily involves a different group I input to the motoneuron pool. When HD depresses transmission at the Ia-motoneuron synapse, a greater afferent volley will be needed to restore the control level of excitation. Given that the EPSP is the balance between Ia excitation and disynaptic (Ib) inhibition, it is likely that the increase in the Ia afferent volley would need to be greater than the depression of transmission at the Ia–motoneuron synapse. Of course, this would be an issue only if the inhibitory circuit did not undergo HD and that may not be the case (Hammar et al. 2002).

HD studied with threshold tracking

The novel feature of this study was the ability to investigate the effects of stimulus trains at different rates on the reflex response of a fixed population of motoneurons. In previous studies, HD has been induced using a variety of conditioning stimuli that activated the same afferents as used by the test H reflex: muscle stretch, tendon percussion, voluntary contraction, a preceding H reflex or, as in this study, stimulus trains of variable rate, i.e., so-called low-frequency depression (Crone and Nielsen 1989; Hultborn and Nielsen 1998; Hultborn et al. 1996; Lamy et al. 2005; Meunier et al. 2007). The major differences are that in these studies 1) HD was maximal at lower stimulus rates than usually reported and 2) the extent of HD depends on the size of the test H reflex.

The extent of change produced by HD measured using threshold tracking is smaller than that reported using the percentage change in reflex amplitude (Fig. 5, see also Burke et al. 1989; Crone and Nielsen 1989; Hultborn and Nielsen 1998; Hultborn et al. 1996). However, the sensitivity of the method for showing HD depends on the variability of the measure in addition to the percentage change. Figures 1B, 1C, and 5C shows that the variability of the threshold for an H reflex of fixed size is much less than the variability of a reflex response to a fixed stimulus, and the coefficient of variation was 20 times less for the threshold tracking technique.

It has been suggested that motoneurons determine the properties of the afferent terminal and thus influence synaptic connectivity (Honig et al. 1983). It would be expected that there would be differences in the extent of HD operating on the afferent projections to low-threshold and high-threshold mo-
toneurons. Previous studies present evidence that small reflexes are more susceptible to low-frequency depression than large reflexes (Cook 1968; Field-Fote et al. 2006; Floeter and Kohn 1997; Lloyd and Wilson 1957; Rossi-Durand et al. 1999; Van Boxtel 1986), in keeping with the greater susceptibility of small reflexes to facilitation or inhibition (Crone et al. 1990; Meinck 1980). However, a number of factors determine this susceptibility, and it cannot be construed as evidence that HD is necessarily greater on the afferent projection to low-threshold motoneurons. This study presented evidence that the opposite is true: that the afferent projection to high-threshold motoneurons is more susceptible to HD. This is intuitively reasonable because lesser depression of Ia synaptic effects on low-threshold motoneurons would render those motoneurons more responsive to the same Ia volley. Given the relatively small test reflex in this study, this would also explain the lower rate dependence seen here than in other studies of low-frequency depression of the soleus H reflex (Burke et al. 1989; Calancie et al. 1993; Cook 1968; Floeter and Kohn 1997; Hoehler and Buerger 1983; Hoehler et al. 1981; Ishikawa et al. 1966; Meunier et al. 2007; Rossi-Durand et al. 1999; Rothwell et al. 1986; Schindler-Ivens and Shields 2000; Van Boxtel 1986).

Functional significance of HD of the H reflex

The functional relevance of HD of the H reflex is controversial. Stein et al. (2007) have argued that HD of the H reflex is an epiphenomenon because it is largely abolished during voluntary contractions. However, this begs the question whether the absence of reflex depression during voluntary contractions means that the underlying mechanism, presumably transmitter depletion (Hultborn et al. 1996), is depressed during voluntary activity or merely offset by other changes. During voluntary activity, the contraction-associated increase in Ia discharge would lead to HD being tested under “adapted” already depressed conditions, so that any reflex depression might not be apparent when the test H reflex is enhanced by a shift in the excitability of the motoneuron pool and depression of Ib inhibition. In addition, as mentioned earlier, depressed Ib activity would alter the likelihood of discharge of higher-threshold motoneurons, the very ones more susceptible to HD. The reduced low-frequency depression of the H reflex in spasticity could be explained by similar “offsetting” mechanisms.

We favor the view that HD operates as a modulating influence on transmission from Ia afferent terminals and presume that it occurs not only on the Ia-motoneuron terminals but possibly also on terminals to ascending pathways and in higher connections. It cannot be assumed that postactivation depression of the synaptic actions of the same afferents are the same at different locations (Hammar et al. 2002), but, nevertheless, HD could be important in proprioception and in the supraspinal adjustments to movement control. Considering just the Ia-motoneuron synapse, it should be appreciated that few muscles are constantly active; most cycle between activity and rest, particularly during tasks such as locomotion, and there is evidence that HD might be biologically important during locomotion (Voigt and Sinkjaer 1998). As noted earlier, HD is depressed in spasticity, and may be restored in parallel with a functional improvement in locomotion in paraparesis (Trimble et al. 1998).

GRANTS

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REFERENCES


