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RESEARCH ARTICLE

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Diurnal changes in the amplitude of the Hoffmann reflex in the human soleus but not in the flexor carpi radialis muscle

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Abstract Changes in the reflex amplitude throughout the day have been observed in non-human mammals. The present experiment tested whether diurnal fluctuations also occur in humans. Hoffmann reflex (*H*-reflex) amplitude was measured in soleus and flexor carpi radialis (FCR) muscles from the data collected over a 12-h period between 7:00–9:00 a.m. and 7:00–9:00 p.m. At 4-h intervals, *M*/*H* recruitment curves were obtained, and two measures of *H*-reflex excitability were calculated. The maximal *H*-reflex (H_{max}) was calculated as the average of the three largest *H*-reflexes. *H*-reflexes were also sampled from the ascending limb of the *M*/*H* recruitment curve (H_A, $n = 10$), with a corresponding *M*-wave of 5% M_{max}. All values were normalized to the maximal *M*-wave (M_{max}). Soleus *H*-reflex amplitude and plantar flexion maximal voluntary isometric contraction force (MVIC) were significantly smaller ($p < 0.05$) in the morning ($H_{max} = 57.2\%$ M_{max}, $H_A = 42.3\%$, M_{max}, MVIC = 162.1 Nm) than in the evening ($H_{max} = 69.1\%$ M_{max}, a 20.1% increase, $H_A = 54.1\%$ M_{max}, a 27.4% increase and MVIC = 195.8 Nm, a 20.8% increase). In contrast, FCR *H*-reflex amplitude and FCR MVIC were unchanged across all testing sessions. The data show that diurnal fluctuations are present in the amplitude of the human soleus but not in the FCR *H*-reflex. Diurnal fluctuation in the human soleus *H*-reflex amplitude must be considered when interpreting *H*-reflex data, especially when a repeated measures design spanning several days is utilized.

Keywords Diurnal rhythm · Hoffmann reflex · Isometric contraction · Human

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Introduction

Diurnal fluctuations have been identified in a range of processes in mammals, including behavioral (Stephan and Zucker 1972), hormonal (Kalsbeek et al. 2003) and neural (Wolpaw and Seegal 1982). Diurnal rhythms in mammalian reflex amplitude have been identified primarily in the soleus muscles of rats (Chen and Wolpaw 1994, 1995; Chen et al. 2002) and the primate biceps brachii (Wolpaw and Seegal 1982; Wolpaw et al. 1984) and soleus muscle (Dowman and Wolpaw 1989) of primates. *H*-reflex amplitude in the rat is largest in the late morning and smallest around midnight (Chen and Wolpaw 1994, 1995). In contrast, the primate *H*-reflex and spinal stretch reflex amplitude are largest at midnight and smallest in the morning (Dowman and Wolpaw 1989). This difference between rats and primates has been ascribed to rodents being nocturnal. Diurnal changes in the *H*-reflex indicate that CNS activity and perhaps reflex function varies with the time of the day. If present in humans, this must be taken into account during experimental and clinical reflex testing. That is, it would necessitate the careful timing of experiments in which *H*-reflexes are evoked as well as in clinical examination in which reflexes are evoked to probe the nervous system. The purpose of this study was to investigate diurnal variations in *H*-reflex amplitude and isometric force production for the soleus and FCR muscle of the human participants.

Methods

Data were collected from thirteen (six females and seven males) participants (age range 22–42 years). Nine

participated in the initial experiment and four participants were involved in a subsequent experiment involving a Latin square design. There were no differences between genders. The data for all 13 subjects were combined as described below. All participants gave their informed consent prior to inclusion in the study. All testing was conducted according to the protocols approved by the Human Research Ethics committee at the University of Victoria and the University of Alberta. Testing commenced 2–3 h after waking for all the participants. The participants were instructed not to consume caffeine 12 h prior to the experiment and to abstain from caffeine consumption for the duration of the experiment to eliminate the influence of caffeine on spinal reflex excitability (see Walton et al. 2003). Physical activity of the participants was limited to light walking (estimated to be $\approx < 5$ km/h), typing and writing (i.e., office work) for the duration of the experiment.

Four testing sessions were completed over a 12-h period with the first occurring between 7:00 and 9:00 a.m. Subsequent testing sessions were conducted at 4-h intervals. During each testing session, data for M/H recruitment curves were obtained for the soleus and FCR, as well as maximal isometric plantar flexion and maximal isometric FCR contraction.

During soleus H-reflex and plantar flexion MVIC data collection, the participants were seated in a chair with their backs supported. Hip, knee and ankle angles were approximately 90, 150 and 90°, respectively. Restraints were placed around the foot to minimize movement. During FCR H-reflex and wrist flexion MVIC collection participants were seated in the same manner. However, in addition, the right arm was supported with 90° of flexion at the elbow and 90° of abduction at the shoulder with the forearm parallel to the floor. The temperature, noise and lighting in the laboratory were similar for all the sessions.

Plantar flexion MVIC values were established with a strain gauge (Omegadyne Ltd., UK Model 101–500, range 0–226.7 kg) and amplified by a custom-made high gain amplifier. Voltage was displayed using custom-written acquisition software utilizing LABVIEW (National Instruments, USA) Force was calculated from each MVIC by converting voltage output into Newtons (1.00 V = 444.39 N). Plantar flexion force was consistently applied with a moment arm length of 0.15 m (measured from the adjustable heel block to the center of the strain gauge). Two methods were used to assess changes in FCR activation throughout the day. The Rehabilitation Neuroscience Laboratory at the University of Victoria used a handgrip dynamometer (Lafayette, model 78010) and the Human Neurophysiology Laboratory at the University of Alberta used a strain gauge (Interface Ltd., Atlanta, GA, USA, Model SSM100, range 0–50 kg) to measure maximal wrist flexion torque (see Ref. Carroll et al. 2005). Both methods were equivalent for the purpose of assessing changes in the maximal voluntary force production in the same subjects.

Electromyography (EMG) was recorded with bipolar surface recording electrodes. EMG signals were pre-amplified and band pass filtered at 30–300 Hz. EMG was collected from the soleus, tibialis anterior, vastus lateralis and bicep femoris muscles during tibial nerve stimulation. Due to the drying of the electrode gel throughout the day, some participants needed to have the electrodes replaced. Therefore, the skin of each site was carefully marked to ensure similar placement of replacement electrodes. During median nerve stimulation, EMG was recorded from FCR, extensor carpi radialis and biceps brachii. Data were sampled at 2,000 Hz with a 12-bit A/D converter. For each M/H curve, 50 sweeps of data were collected 20 ms before and 90 ms after the stimulus was delivered. The majority of the data were collected between H_{min} (the minimal attainable peak to peak amplitude of the H-reflex) and H_{max} (the maximal attainable peak to peak amplitude of the H-reflex). However, the distribution of samples in the recruitment curve was not uniform since more H-zreflex data points were purposely collected at 5% M_{max}. M_{max} measurements were obtained from the M/H recruitment curve while the participants maintained a tonic background contraction equivalent to 10% of their maximal EMG output. This background EMG level was equivalent to 10% of the MVIC. The EMG signal was low pass filtered at 3 Hz and displayed on an oscilloscope to provide constant visual feedback to the participants. The interstimulus interval varied randomly between 3 and 5 s. The order of testing was randomized for tibial and median nerve stimulation for each subject. The tibial and median nerves were stimulated with single 1 ms square-wave pulses delivered over the popliteal fossa and proximal to the medial epicondyle of the humerus, respectively. The participants maintained a 10% background EMG contraction during soleus and FCR H/M recruitment curve data collection according to a displayed target force attained from the MVIC. The M-waves and H-reflexes of each participant were normalized to the corresponding M_{max} taken at that time (see Zehr 2002).

Two measures of H-reflex excitability were calculated from each M/H recruitment curve: H_{max} and H_A. H_{max} was calculated as the average of the three largest H-reflexes. H_A was calculated as the average of ten H-reflexes obtained from the ascending limb of the M/H recruitment curve, with a corresponding M-wave of 5% M_{max}. M_{max} was taken to be the single largest M-wave. All M-wave and H-reflex data were calculated from peak-to-peak amplitude. All torques were calculated from the largest MVIC.

Maximal isometric plantar flexion and wrist flexion MVIC was recorded during each testing session. The participants performed 3, 3–5 s MVICs with at least 60 s rest between the contractions.

Latin square design

A 4×4 Latin square experiment examining the soleus *H*-reflex and MVIC response was performed to examine the potential serial ordering effects. Four subjects (two females and two males) not involved in the original study were randomly assigned to one of the four testing sessions (9:00 am, 12:00 pm, 3:00 pm and 6:00 pm) for each day in a 4-day testing protocol (Monday–Thursday). Methods of data collection and analysis were identical to those described above. Due to the small sample size of our 4×4 Latin square experiment, the probability levels were not expected to be comparable to the main experiment. Thus, we compared the effect sizes between our two experiments, (see Refs. Cohen 1988; Rosnow and Rosenthal 1996).

Statistics

Separate one-way repeated measures analyses of variance tests (ANOVA) were used for soleus and FCR data to examine the effects of time of day on the dependant variables (H_{max}, H_A and MVIC). The significance level was set at $p < 0.05$. Tukey's HSD tests were used for post hoc comparisons ($p < 0.05$) if the ANOVA results indicated a significant main effect. All statistics were performed using absolute values normalized to percent M_{max}, with the exception of torque. Figures 2a, b are reported as percentage change scores for clarity. All the data are presented as mean ± SD.

Results

H-reflex amplitude

First testing session values for soleus H_A and H_{max} averaged 42.3%M_{max} and 57.2%M_{max}, respectively. Soleus H_A values increased 27.4% between the first and fourth testing sessions ($p = 0.01$). Soleus H_A values in the fourth testing session (54.1%M_{max}) were also significantly higher ($p = 0.04$) than those in the second session (42.6%M_{max}), but not the third session (43.9%M_{max}). Soleus H_{max} values followed a similar diurnal pattern with a 20.1%M_{max} increase ($p = 0.03$) between the first and fourth (69.1%M_{max}) session (see Figs. 1, 2a and Table 1).

Testing values from the first session for FCR H_{max} and H_A were 38.9%M_{max}, 20.1%M_{max}, respectively. FCR H_A and H_{max} values did not fluctuate significantly throughout the day; however, there was an initial increase in H_A values, which then plateaued (see Fig. 2b and Table 1). *M*-waves and background EMG remained unchanged ($p < 0.05$) between testing sessions for soleus and FCR.

H-reflex gain

The linear fit of the least sum of squares regression between the soleus *H*-reflex amplitude and *M*-wave showed a steady increase in the slope from the first to the fourth testing session (first slope 7.1 ± 5.4; second slope 8.6 ± 3.2; third slope10.5 ± 8.6; fourth slope 13.4 ± 10.4; see Fig. 3, 4. However, significant differences were identified only between the slope of the first and fourth testing session ($p = 0.05$). Linear regression trend line data for one subject are shown in Fig. 3.

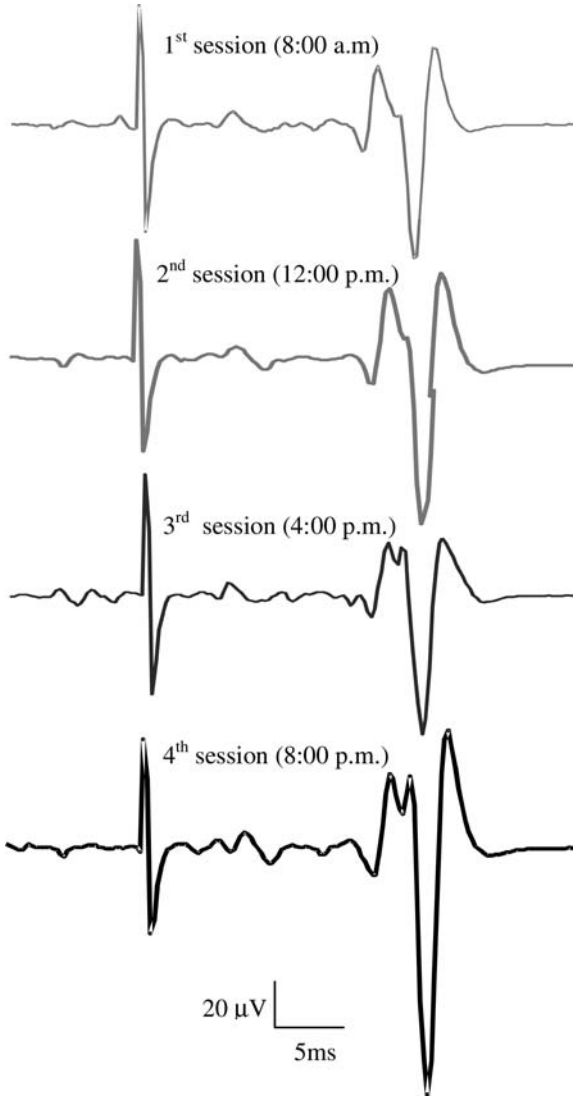

Fig. 1 Soleus *H*-reflex increases in amplitude from morning to evening. The large artifact can be seen at the far left of the sweeps with the *M*-wave following as the small middle trace, and finally the clear *H*-reflex waveform shown at *right*. Data are from a single subject

Maximal voluntary isometric contractions

A significant 20.8% increase ($p = 0.01$) in plantar flexion MVIC occurred between the first (162.1 Nm) and the fourth (195.8 Nm) testing sessions (see Fig. 2a). Also, the increase in plantar flexion MVIC from the second (166.8 Nm) to the fourth testing session was also significantly larger ($p = 0.03$). Wrist flexion MVIC values

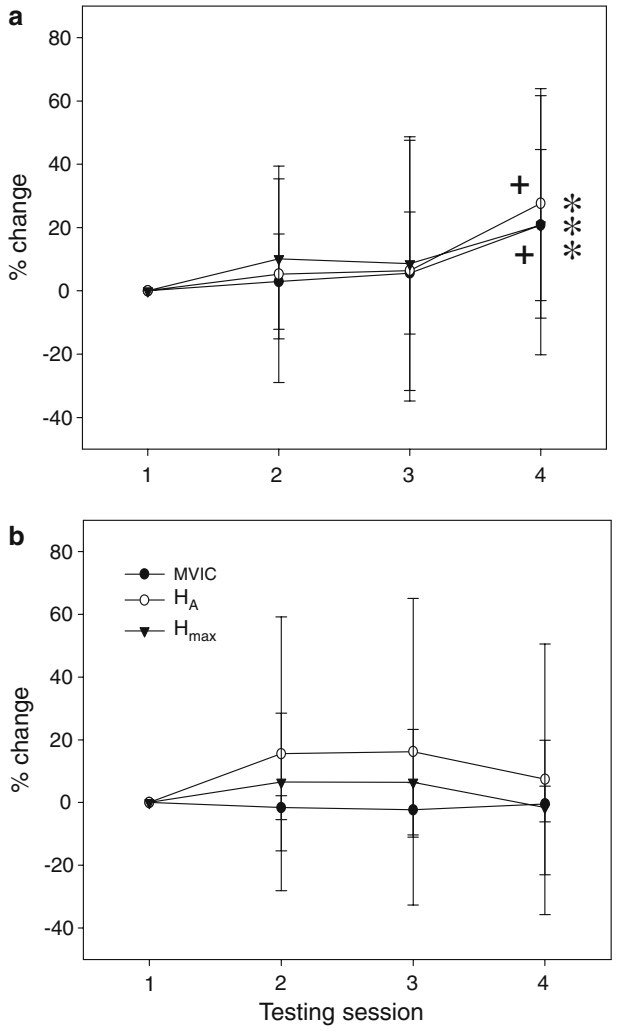

Fig. 2 **a** Percentage change between testing session 1 (7:00 and 9:00 a.m.), 2 (11:00 and 1:00 p.m.), 3 (3:00 and 5:00 p.m.) and 4 (7:00 and 9:00 p.m.) for plantar flexion MVIC (*solid circle*) soleus H_{max} (*triangle*), and soleus HA (*open circle*). Data points were averaged across the group ($n=13$). *Asterisks* (*) indicates significant differences from session 1. *Crosses* (+) indicate significant differences from session 2 **b** Percentage change between testing session 1 (7:00 and 9:00 a.m.), 2 (11:00 and 1:00 p.m.), 3 (3:00 and 5:00 p.m.) and 4 (7:00 and 9:00 p.m.) for wrist flexion MVIC (*solid circle*), FCR H_{max} (*triangle*) and FCR H_A (*open circle*)

were not significantly different between the sessions (see Fig. 2b).

Effect sizes

Cohen's d values of soleus *H*-reflex data were similar in magnitude when compared to our 4×4 Latin square design (see Table 2).

Discussion

The main findings of this experiment were that plantar flexion MVIC and soleus *H*-reflex amplitudes were

Table 1 Group data showing H_{max}, H_A, M_{max} and MVIC values for soleus and FCR for all the testing sessions. All *H*-reflex data is normalized to M_{max} and MVICs are expressed as Newton Meters. M_{max} data is expressed in mV

	8:00 am	12:00 pm	4:00 pm	8:00 pm
Soleus H_{max}				
Mean	57.2	62.9	62.1	69.1*
SD	9.9	15.5	14.8	14.3
Soleus H_A				
Mean	42.3	42.6	43.9	54.1*$^{+}$
SD	13.1	15.7	18.3	13.7
Soleus M_{max}				
Mean	7.9	7.3	7.4	6.6
SD	1.9	2.3	2.5	2.1
FCR H_{max}				
Mean	38.9	41.5	41.4	38.3
SD	22.9	22.2	22.9	23.6
FCR H_A				
Mean	20.1	23.3	23.3	21.6
SD	16.0	13.4	15.8	13.2
FCR M_{max}				
Mean	5.1	5.0	5.3	5.0
SD	2.7	3.1	3.1	2.5
Soleus MVIC				
Mean	162.1	166.8	171.2	195.8*$^{+}$
SD	63.4	71.3	64.1	75.1
FCR MVIC				
Mean	35.8	35.3	35.0	35.7
SD	14.9	14.3	11.7	13.4

Asterisks (*) indicate significant differences from session 1. *Crosses* (+) indicate significant differences from session 2

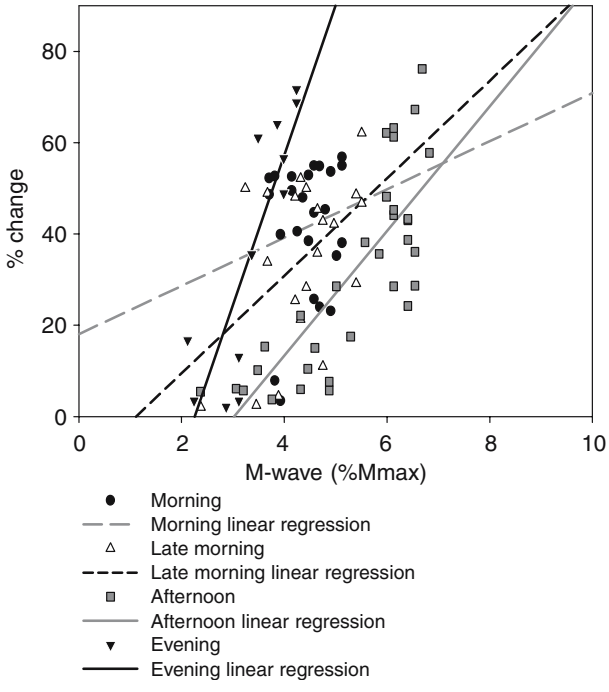

Fig. 3 The soleus *H*-reflex gain expressed as the linear fit of the least sum of squares regression between the *H*-reflex amplitude and *M*-wave for one subject. Data were taken from H_{min} to H_{max} and hence represent the ascending limb of the H/M recruitment curve. For clarity, only data between 0 and 10% M_{max} have been displayed. The slope of the line expresses the gain of the *H*-reflex. The *H*-reflex gain increases from morning to evening

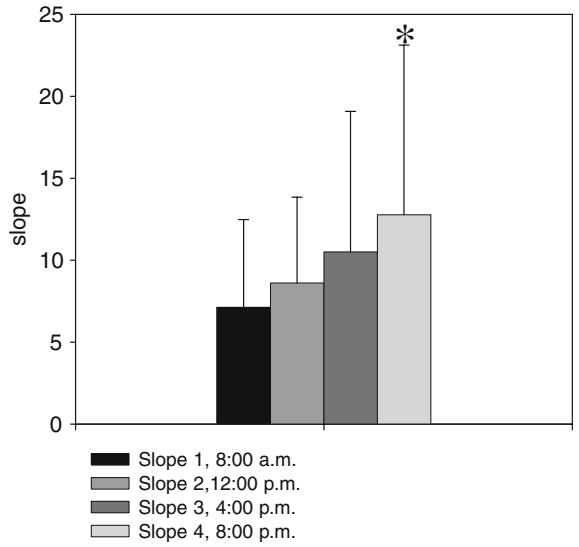

Fig. 4 Mean group slopes of the linear regression line through the ascending limb of the *H*-reflex recruitment curve. *Asterisks* (*) indicate significant differences from session 1

Table 2 Calculated Cohen's d values between sessions 1:2, 1:3 and 1:4

	8:00 am: 12:00 pm (1:2)	12:00 pm: 4:00 pm (1:3)	4:00 pm: 8:00 pm (1:4)
Latin square design			
Cohen's d for H_{max}	0.44	0.11	0.80
Cohen's d for H_A	0.40	0.36	0.80
Longitudinal design			
Cohen's d for H_{max}	0.45	0.5	1.01
Cohen's d for H_A	0.42	0.35	0.96

significantly larger in the evening than in the morning and that the same measures did not change for FCR. Furthermore, the results of our 4×4 Latin square design experiment showed no order effect. Thus, we are confident that there was no serial ordering or learning effects throughout our experiment.

Soleus H_A and H_{max} increased, despite stable background EMG and *M*-wave values (see Fig. 1a). These findings are similar to primate experiments examining diurnal and circadian changes in the amplitude of the spinal stretch reflex (Wolpaw and Seegal 1982) and *H*-reflex (Dowman and Wolpaw 1989). The pattern displayed by the rat, a nocturnal rodent, is just the opposite (Chen and Wolpaw 1994, 1995). Our finding that plantar flexion MVIC increases from morning to evening is in agreement with the previous experiments that have found similar increases in the adductor pollicis muscle of the hand (Martin et al. 1999) and elbow flexors of the arm (Gauthier et al. 1996). However, the wrist flexion MVIC did not change in the present experiment, which is contradictory to these findings. A significant increase in the maximal plantar flexion throughout the day may reflect not only the importance of the triceps surae in locomotion, but also in the maintenance of erect posture. Recent data suggest that standing quietly requires frequent, tiny ballistic adjustments of the calf muscles (Loram et al. 2005). An increase in the size of the soleus *H*-reflex with a simultaneous increase in the force produced by this muscle may be an expression of the activity needed to generate corrective motor responses throughout the day.

The change in soleus *H*-reflex amplitude could be due to altered levels of presynaptic inhibition especially since background EMG and M-amplitude remained stable (Zehr 2002; Misiaszek 2003). Such diurnal changes in presynaptic inhibition could be mediated through altered GABA receptor affinity at the level at the Ia terminal of the α-motoneuron or increased production of GABA in the suprachiasmatic nucleus. The suprachiasmatic nucleus has been suggested to be the site of pacemaker activity in mammals since a diurnal change in feeding and locomotor activity was observed after ablation of this area in primates (Stephan and Zucker 1972). The suprachiasmatic nucleus may alter the production of GABA, thus altering the level of presynaptic inhibition. However, to explain our findings, this would have to selectively influence the α-motoneurons of soleus and not FCR. Chen and Wolpaw (1994) have suggested that the pacemaker responsible for diurnal variations in *H*-reflex amplitude likely exerts its influence presynaptically on the Ia synaptic connection and/or postsynaptically on the α-motoneuron. If this hypothesis is correct, the presynaptic inhibition influence is likely not caused by the humoral pathways, since in our experiment FCR *H*-reflex amplitude did not show any diurnal variation.

That neither H_{max} nor H_A reflex amplitude changed in FCR suggests that diurnal changes in CNS function may not affect reflex pathways for all the muscle groups in a similar manner. A difference in diurnal CNS function between the lower and upper body may be due to the bipedal nature of humans. That we walk upright and make habitual voluntary movements with our hands may predispose us to different CNS activity in the lower versus upper limbs. In this context, fluctuations in lower limb reflex activity may be more relevant for locomotion. Perhaps, an increased reflex excitability is a mechanism to help offset the effects of fatigue experienced by walking throughout the day. Or possibly, from an evolutionary view this could be related to a necessity for escape that changes throughout the day. In contrast, a more consistent excitability of reflex control for the wrist or hand muscles may be anticipated due to the more similar use of the arms throughout the day.

In humans, stretch and *H*-reflexes may be differentially modulated (Morita et al. 1998). If our observations on *H*-reflex fluctuation can be extended to diurnal fluctuations in tendon taps, as shown in the monkey (Wolpaw and Seegal 1982; Wolpaw et al. 1984), it could be an issue to consider for clinicians using tendon taps as part of a neurological examination, however, this point requires further clarification. Regardless of the mechanisms underlying the diurnal fluctuations in soleus

H-reflex amplitude, experimenters utilizing soleus *H*-reflexes are advised to control for the effect of time of day when taking repeated measures or comparing across time within subjects.

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