

Effects of homosynaptic depression on spectral properties of H-reflex recordings

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Effects of homosynaptic depression on spectral properties of H-reflex recordings**Abstract**

The purpose of this study was to determine the effects of homosynaptic depression (HD) on spectral properties of the soleus (SOL) H-reflex. Paired stimulations, separated by 100 ms, were used to elicit an unconditioned and conditioned H-reflex in the SOL muscle of 20 participants during quiet standing. Wavelet and principle components analyses were used to analyze features of the time-varying spectral properties of the unconditioned and conditioned H-reflex. The effects of HD on spectral properties of the H-reflex signal were quantified by comparing extracted principle component scores. The analysis extracted two principle components: one associated with the intensity of the spectra and one associated with its frequency. The scores for both principle components were smaller for the conditioned H-reflex. HD decreases the spectral intensity and changes the spectral frequency of H-reflexes. These results suggest that HD changes the recruitment pattern of the motor units evoked during H-reflex stimulations, in that it not only decreases the intensity, but also changes the types of motor units that contribute to the H-reflex signal.

Introduction

Regulating the excitability of the alpha-motoneuron pool at the spinal level of the central nervous system is essential for normal posture and coordinated movement (Nielsen 2004). A common tool that is used to study the excitability of the alpha-motoneuron pool, and its modulation or regulation, is the electrical analogue of the mono-synaptic stretch reflex, i.e., the Hoffmann or H-reflex (Zehr 2002; Stein and Thompson 2006). The H reflex is not, however, a direct measure of alpha-motoneuron excitability, because its reflex amplitude is significantly influenced by pre- or post-synaptic inhibition (Zehr 2002; Stein and Thompson 2006). It is therefore important for researchers to recognize that the H-reflex represents only a 'net' measure of alpha-motoneuron pool excitability. A further pragmatic consideration is that the influence of pre-synaptic mechanisms on H-reflex amplitude should be considered in experimental paradigms that aim to study alpha-motoneuron pool excitability.

Homosynaptic depression represents a mechanism that is responsible for short-term activity-dependent depression of alpha-motoneuron pool excitability due to prior activation of homonymous IA afferents (Hultborn et al. 1996; Aymard et al. 2000). Although different from classic GABAergic depression that results from pre-synaptic inhibition of IA terminals, homosynaptic depression is still considered a presynaptic regulator of alpha-motoneuron pool excitability (Hultborn et al. 1996). The inhibitory effects of homosynaptic depression are related to a decrease in neurotransmitter release at the level of IA afferent terminals that results from prior activation of homonymous IA afferent fibers (Hultborn et al. 1996). Activation of sensory fibers can result from either a passive or active movement as well as from externally applied mechanical or electrical stimuli (Hultborn et al. 1996; Earles et al. 2002; Earles et al. 2002). The fact that the inhibitory effect of electrically induced homosynaptic depression is altered in

clinical populations (Huang et al. 2006) and appears amenable to training interventions (Guan and Koceja 2011; Hoseini et al. 2011) provides an important clinical rationale for understanding and characterizing the mechanism and outcomes associated with homosynaptic depression.

While the effect of homosynaptic depression on alpha-motoneuron pool excitability has been characterized with respect to H-reflex magnitude, little is known about its influence on the spectral properties of H-reflexes. Spectral properties can provide detailed information about motor unit function across different types of muscular contractions or tasks (Wakeling and Rozitis 2004; Wakeling et al. 2006). Wavelet analysis can be used to assess the time-varying spectral properties of electromyographic signals. When combined with principle component analysis, wavelet analysis can be used to determine the intensity and frequency of myoelectric spectra, which can help identify motor unit recruitment strategies during volitional or evoked contractions (Wakeling and Rozitis 2004). For example, these analyses showed that electrically evoked reflex contractions (i.e., H-reflex) can preferentially recruit faster motor units than mechanically evoked reflex contractions (i.e., Tendon-tap) as evidenced by higher myoelectric frequencies (Wakeling and Rozitis 2004). Collectively, these studies indicate that the combination of principle component and wavelet analyses can effectively capture the time-varying spectral properties of electromyographic signals and delineate between their intensity and frequency content. Application of these methods may therefore be suitable to analyze the effects of electrically induced homosynaptic depression on H-reflex spectral properties. Results from such a study may provide novel mechanistic insight into activity-dependent motor unit function.

The purpose of this study was to determine the effects of homosynaptic depression on spectral properties of the H-reflex. Since, homosynaptic depression decreases the alpha-

motoneuron pool excitability, as measured through a decrease in H-reflex amplitude, it would be reasonable to expect a concomitant decrease in the intensity spectra of the H-reflex. Perhaps of greater interest would be to determine the effect of homosynaptic depression on the frequency content of the spectra, because any such changes would likely implicate altered motor unit recruitment. We thus hypothesized that wavelet and principle component analyses of the unconditioned and conditioned H-reflex would identify changes in spectral properties that reflect the effects of homosynaptic depression on motoneuron function consistent with 1) a lower spectral intensity, and 2) altered spectral frequency.

Materials and Methods

Subjects

Twenty (11 males, 9 females) recreationally active, healthy young adults (Mean±SD: 27.4±4.4 years) with no known neurological deficits were recruited for this study. All participants provided written informed consent approved by the University's Institutional Review Board before data collection.

Experimental Protocol

To determine the effects of homosynaptic depression on spectral properties of H-reflexes, a set of paired stimulations with equal intensity were delivered to a mixed peripheral nerve. These stimulations were used to elicit a 'normal' or unconditioned H-reflex and a conditioned H-reflex in the soleus muscle of participants during quiet standing. Stimulations were delivered every 15 seconds to minimize the effects of homosynaptic depression between subsequent sets of paired stimulations. A total of 10 sets of paired stimulation were recorded for each subject.

Electromyography

The surface electromyogram (EMG) from the soleus muscle was recorded at a frequency of 2000 Hz with lubricated Ag/AgCl surface-recording electrodes (BIOPAC Systems Inc., Goleta, CA). The surface electrodes were placed serially along the midline of the soleus with an inter-electrode distance of approximately two centimeters. A stimulating electrode (2 cm²) was placed in the popliteal fossa in the back of the knee and a carbon-rubber dispersal electrode (3 cm²) was placed on the distal thigh just superior to the patella. H-reflex responses were elicited with an s88 Grass stimulator (Grass Technologies, West Warwick, RI) that delivered a 1 ms square wave pulse to the tibial nerve while participants were standing quietly with hands hanging at the side and knees slightly flexed. An abbreviated recruitment curve was used to determine the maximum H-reflex and M-wave. The stimulation intensity was then carefully adjusted to produce an H-reflex equivalent to 10 % of the maximum M-wave and remained at this setting throughout the study. A second stimulation of equal intensity was then added to the experimental protocol. The two paired stimulations were separated by a 100 ms inter-stimulus interval (Figure 1a). The first stimulation thus produced a normal or unconditioned H-reflex response that represents basic motoneuron pool excitability, whereas the second stimulation produced a conditioned response that reflected the rate-dependent inhibition of the IA afferent- α -motoneuron synapse due to the prior activation from the first stimulation.

Insert Figure 1 about here

Data processing

H-reflex magnitudes were determined from the peak-to-peak magnitude from the unconditioned and conditioned EMG recordings. A wavelet analysis of the EMG recordings and a subsequent

principle component analysis were used to determine spectral properties of the normal and conditioned H-reflex recordings from each individual for all ten trials. Preliminary pilot data suggested that stimulation artifact confounded the results from the wavelet analysis. All trials were therefore trimmed to exclude the first 20 ms after stimulation of all EMG recordings to limit the influence of these confounders. Subsequently, two sets of 50 ms samples of EMG data, which spanned the time interval from 20-70 ms after the first and second stimulation, were used for all remaining analyses.

The EMG data was resolved into the time-frequency domain with a wavelet technique developed in MATLAB (The MathWorks, Inc. Natick, MA) at the University of Colorado (<http://atoc.colorado.edu/research/wavelets>). This algorithm uses a Morlet wavelet to calculate the time-varying intensity across frequencies for the duration of the EMG signal (Figure 1b). The calculated time-varying intensity is then summed across all frequencies into a global wavelet spectrum. The global wavelet spectra for the ten unconditioned and ten conditioned H-reflex were then averaged into two sets of ensemble averages for each individual. The two ensemble average recordings were then transposed and pooled into data matrix **A** that consisted of 40 rows (20 subjects x 2 ensemble averages per subject [i.e., one normal H-reflex + one conditioned H-reflex]) and 29 columns (arbitrary number of down-sampled frequencies). A principle component analysis was then used to extract the unit eigenvectors and eigenvalues through orthogonal matrix decomposition from the covariance matrix **B** of the original data matrix **A** (Wakeling and Rozitis 2004; Wakeling et al. 2006). Eigenvectors (i.e., principle components) were retained and further analyzed if the associated eigenvalues for a given eigenvector were greater than one (i.e., unity). Extracted eigenvectors were then multiplied with the ensemble average global wavelet spectra from each individual's normal and conditioned H-reflex. The

summation of the individual multiplication products between the eigenvectors and the global wavelet spectra then gave a set of principle component scores that determined how much of each eigenvector (i.e., principle component) was present within a given global wavelet spectrum (Wakeling and Rozitis 2004; Wakeling et al. 2006).

Statistical analysis

Preliminary analyses indicated unequal variances and violation of the normality assumption. Non-parametric tests were therefore chosen for further inferential statistical analyses. The Wilcoxon-Signed Rank Test for comparison of two-related samples was used to test for differences between peak-to-peak H-reflex magnitude and principle component scores from unconditioned and conditioned recordings. The alpha-level for significance testing was set at 0.05. All analyses were run in SPSS Statistics 17.0 (IBM Corporation, Somers, NY, USA).

Results

The conditioned H-reflex recordings were significantly depressed ($p < 0.001$) compared to the unconditioned H-reflex recordings (Table 1).

The PCA extracted two principle components that cumulatively explained 98.8% of the variance in the global wavelet spectra data (i.e., spectra pooled from the unconditioned and conditioned H-reflex data). The first principle component accounted for 91.7% of the variance in the global wavelet spectra and had positive weightings across all frequencies, whereas the second extracted principle component accounted 7.1% of the variance in the global wavelet spectra and transitioned between a negative and positive weighting (Figure 2). It therefore appeared that the first principle component captured the shape and magnitude of the global wavelet spectra and the

second principle component captured a shift in the frequency content of the global wavelet spectra.

Insert Figure 2 about here

Principle component scores differed significantly between unconditioned and conditioned H-reflexes (Table 1). The scores for the first principle component were significantly smaller ($p = 0.001$) for the unconditioned H-reflex than the conditioned H-reflex. In addition, the scores for the second principle component were also significantly smaller ($p = 0.004$) for the unconditioned H-reflex than the conditioned H-reflex.

Insert Table 1 about here

Discussion

The purpose of this study was to determine the effects of homosynaptic depression on spectral properties of H-reflexes in the soleus muscle. We hypothesized that homosynaptic depression would 1) decrease spectral intensity and 2) alter spectral frequency of a conditioned H-reflex when compared to an unconditioned H-reflex. The results of this study confirmed both original hypotheses in that homosynaptic depression decreased the spectral intensity and frequency of a conditioned H-reflex when compared to an unconditioned H-reflex.

The stimulation protocol used in the current study was associated with a depression in the peak-to-peak magnitude of the conditioned H-reflex. The decrease in the magnitude of the H-reflex in response to paired stimuli was approximately 80%, which is well in line with other studies (Earles et al. 2002). The decrease in H-reflex magnitude of the second compared to the first out of the two stimuli arises from prior activation of homonymous IA afferents. This inhibition is thought to act as a presynaptic regulator and gate motoneuron pool excitability.

The decrease in peak-to-peak magnitude of the conditioned H-reflex was also accompanied by a decrease in spectral intensity, which likely indicates an analogous decrease in alpha-motoneuron pool excitability. The spectral intensity of an EMG signal is positively related to force output during voluntary isometric contractions or electrically evoked twitch contractions (Wakeling and Rozitis 2004). Since the mechanical manifestation of an H-reflex can be characterized by the force output of its twitch response it is likely that the decrease in spectral intensity also points to a smaller muscle response. A smaller twitch response would point to reduced H-reflex amplitude, which is consistent with the effects of homosynaptic depression on alpha-motoneuron pool excitability and H-reflex recordings (Aymard et al. 2000),

The combination of wavelet and principle component analyses has also been demonstrated to be sensitive enough to discern between recruitment strategies of different motor units (Wakeling and Rozitis 2004; Wakeling et al. 2006; Lee et al. 2011). Specifically, these methods can detect preferential recruitment of faster motor units during rapid shortening of muscle fascicles (Wakeling and Rozitis 2004; Wakeling et al. 2006). For example, spectral intensity increased as pedaling cadence increased at low torques, and changes in the ratio between spectral intensity and frequency were associated with changes in muscle fascicle strain rates during pedaling (Wakeling et al. 2006). The results of the current study show that that homosynaptic depression decreased H-reflex spectral frequency, when conditioned by prior homonymous activation of IA afferents. Since homosynaptic depression is associated with a decrease in neurotransmitter release (Hultborn et al. 1996), a change in motor unit recruitment is also to be expected. In particular, a drop in neurotransmitter release would decrease the excitatory postsynaptic potential, which would effectively diminish motoneuron depolarization and reduce the number of motor units activated during the conditioned H-reflex. It therefore appears that homosynaptic

depression changes the recruitment pattern of the motor units evoked during H-reflex stimulations. This finding further illustrates the novelty of information gained from the combination of wavelet and principle component analyses, because these methods were able to detect physiological changes associated with homosynaptic depression beyond a change in H-reflex magnitude.

A few general limitations related to the methods of this study should be considered when interpreting the current results. Homosynaptic depression is dominant in low-threshold motor units (Hultborn et al. 1996). Furthermore, the sensitivity of the H-reflex to conditioning inputs depends on H-reflex size (Crone et al. 1990). It could therefore be surmised that the results of this study could differ if the intensity of the stimulation were increased so as to alter the IA afferent recruitment, which in turn would also alter the recruitment of motoneurons. In addition, H-reflex magnitude and homosynaptic depression were assessed during quiet standing posture. The amount of IA afferent activation, however, changes with postural sway and depends on whether a muscle is actively shortening or lengthening (Tokuno et al. 2008). Passive stretch of a muscle, which may occur during postural sway, may also affect the amount of homosynaptic depression (Hultborn et al. 1996). Postural equilibrium reactions in response to repeated nerve stimulations may also have affected the H-reflex magnitude and homosynaptic depression. While the stimulation intensities in the current study were small ($\sim 10\%$ of M_{\max}) and their influence on postural sway may have been negligible, other postures, such as sitting or lying, may have been preferable to limit such reactions. Although these limitations could represent potential confounders to the results of the current study, the intraclass reliability of experimental protocols that measure homosynaptic depression during quiet standing is very high (> 0.90) when sufficient number (> 4) of stimulations is used (Earles et al. 2002). The use of ten low-intensity

stimulations in this study should therefore help limit the effects of postural sway or postural equilibrium reactions on either H-reflex magnitude or homosynaptic depression.

In conclusion, the results from this study indicate that homosynaptic depression decreased the spectral intensity and changed the spectral frequency of H-reflexes. Homosynaptic depression affects motor unit output during electrically evoked reflex contractions, and changes the recruitment pattern of the motor units evoked during H-reflex stimulations in that it decreases the intensity and changes the types of motor units that contribute to the H-reflex signal. These changes are consistent with previously described effects of homosynaptic depression, but extend findings beyond the magnitude of H-reflex, because it also showed, for the first time, a change in the intensity and frequency of the spectra, which collectively indicate a change in motor unit recruitment.

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Declaration of Interest: The authors report no conflict of interest.

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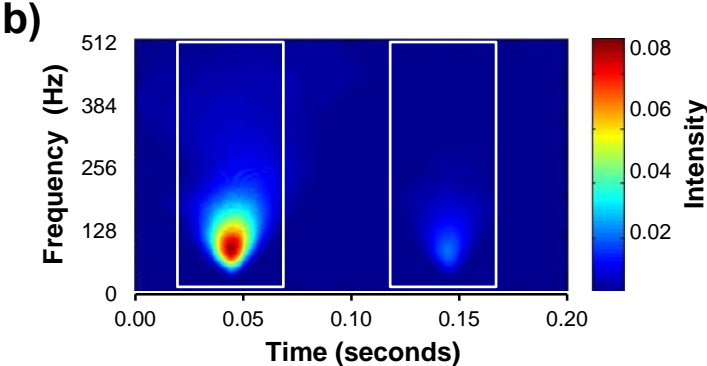
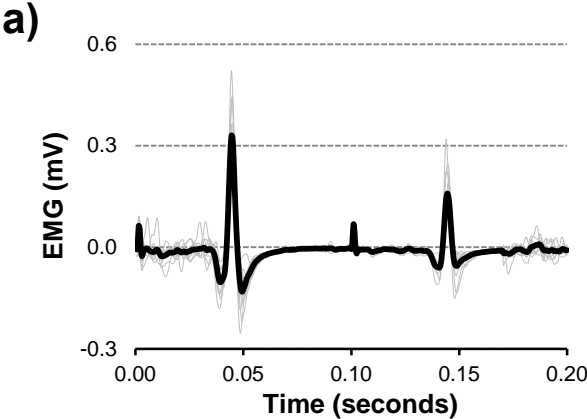
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Figure Legends

Figure 1. Standard electromyographic recordings and time-frequency spectra of H-reflex recordings. **a)** Ten paired H-reflex recordings (thin grey lines) and ensemble average (thick black line) for one individual. The first H-reflex that occurs around 55 ms represents the unconditioned recording and the second H-reflex that occurs 100 ms later represents the conditioned recording. **b)** Time-frequency of paired H-reflex recordings after wavelet transformation. The white boxes around the time-frequency spectra represent the data that were used for analysis in order to eliminate the influence of the stimulus artifact.

Figure 2. Principal component (PC) and vector representation of EMG time-frequency spectra from H-reflex recordings. **a)** Weighing for the first and second PC. **b)** Ensemble average of the spectral intensity for the conditioned and unconditioned H-reflexes. **c)** Vector representation of the PC loading scores for the time-frequency spectra (black data = unconditioned H-reflex; grey data = conditioned H-reflex).



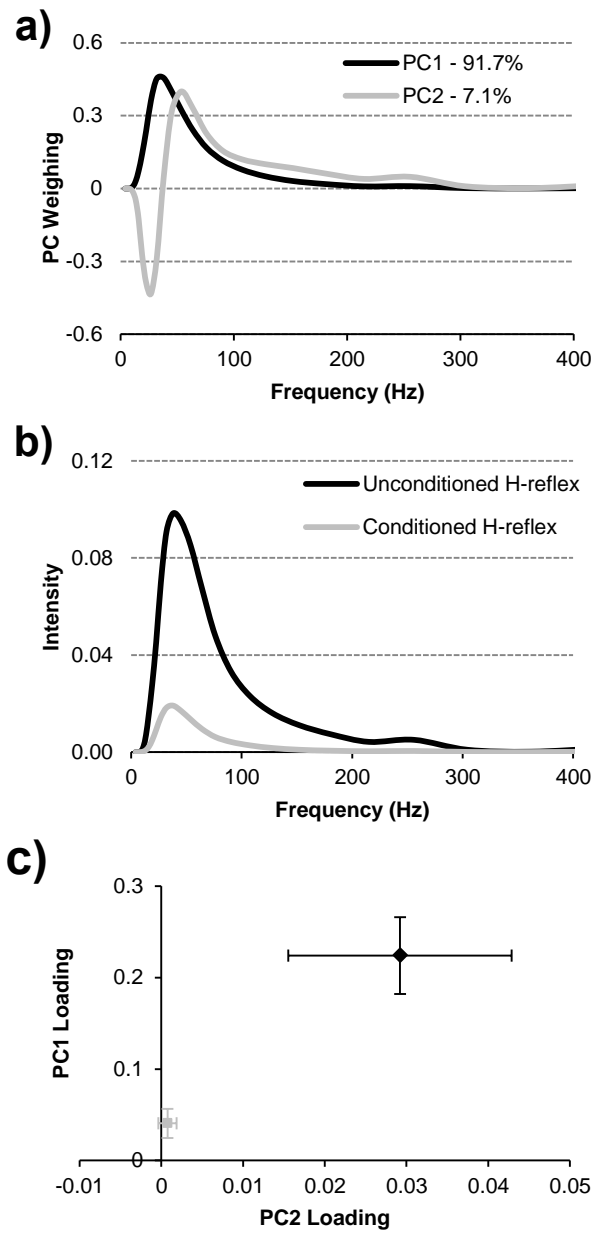


Table 1.

Unconditioned and conditioned H-Reflex magnitudes and principal components scores. Data are presented as Mean \pm SD.

	Unconditioned	Conditioned
H-Reflex [*]	0.66 \pm 0.30	0.15 \pm 0.17
PC1 Score [*]	0.1914 \pm 0.1836	0.0021 \pm 0.0036
PC2 Score [*]	0.0417 \pm 0.0512	0.0010 \pm 0.0014

^{*} $p < 0.05$ Unconditioned vs. Conditioned