Functional principal component analysis of H-reflex recruitment curves

Kristof Kipp [a,b,*], Samuel T. Johnson [b], Mark A. Hoffman [b]

[a] Department of Physical Medicine and Rehabilitation, University of Michigan, Ann Arbor, MI 48109, United States
[b] Department of Nutrition and Exercise Sciences, Oregon State University, Corvallis, OR 97331, United States

ARTICLE INFO

ABSTRACT

The primary purpose of this study was to use functional principal component analysis (FPCA) to analyze Hoffman-reflex (H-reflex) recruitment curves. Smoothed and interpolated recruitment curves from 38 participants were used for analysis. Standard methods were used to calculate three discrete variables (i.e., H_{max}/M_{max} ratio, H_{th}, H_{slp}). FPCA was then used to extract principal component functions (PCFs) from the processed recruitment curves. PCF scores were calculated to determine how much each PCF contributed to an individuals' recruitment curve. The analysis extracted three PCFs, and three sets of PCF scores. Correlation analyses and systematic variation in the PCF scores indicated that the scores for the first PCF were primarily correlated to H-reflex threshold (H_{th}) and that the scores for the second and third PCFs were correlated to H-reflex magnitude (H_{max}/M_{max} ratio) and slope (H_{slp}), respectively. In addition, results from the FPCA indicated that the first PCF explained 56.0% of the variance between all H-reflex recruitment curves, whereas the second and third PCFs explained 24.1% and 13.0%, respectively. The high correlations indicate FPCA-derived PCFs capture similar physiological information as the standard discrete variables and suggest that application of FPCA to H-reflex recruitment curves could be used in future studies to complement traditional analyses that investigate excitability of the motoneuron pool.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

An essential feature of central nervous system function is the regulation of the excitability of the motoneuron pool at the spinal level (Nielsen, 2004). Arguably, the most commonly used tool to investigate motoneuron pool excitability is the Hoffmann or H-reflex (Capaday, 1997; Tucker et al., 2005; Zehr, 2002). The most basic protocol used to study the H-reflex is an electrical stimulation protocol that uses a series of progressively increasing stimulation intensities to develop an H-reflex recruitment curve (Stein and Thompson, 2006; Zehr, 2002).

In order to quantify motoneuron pool excitability, researchers typically extract several discrete variables from H-reflex recruitment curves (Funase et al., 1994a, 1994b; Knikou et al., 2009). Most often, the peak of the H-reflex recruitment curve (i.e., peak H-reflex recording) is measured and compared to the maximum motor response (M-wave). The ratio between the H-reflex and M-wave represents the percentage of depolarized motoneurons in response to Ia-afferent activation (Tucker et al., 2005; Zehr, 2002). Another discrete variable that is often measured is the peak slope of

the H-reflex recruitment curve, which is used to estimate the rate of change in motoneuron pool excitability in response to the increase of Ia-afferent input to the motoneuron pool (Christie et al., 2004; Funase et al., 1994a; Hayes et al., 2009). A third discrete variable, which has also been used to estimate the excitability of the lowest threshold motoneurons, is the stimulation intensity or threshold where the H-reflex first appears and the H-reflex recruitment curve begins to increase (Funase et al., 1994b). Although, studies frequently use all three discrete variables, a valid question that arises is whether they adequately capture information from a continuous curve that consists of numerous observations (i.e., an H-reflex recruitment curve).

Functional principal components analysis (FPCA) is a multivariate statistical analysis that can be used to explore data where observations consist of continuous functions (Ramsay and Silverman, 1997). FPCA uses a data reduction method to produce a small set of principal component functions (PCFs), which captures features that characterize the salient aspects contained within the original data set (Ramsay and Silverman, 1997). An increasing number of researchers have used FPCA and found that PCFs effectively captured features that characterize muscle activation strategies or joint kinematics during movement (Chester, 2009; Daffertshofer et al., 2004; Ramsay and Silverman, 1997). In addition, these studies generally report that PCFs provide more helpful information than traditional discrete variables when data sets consist of continuous functions.

* Corresponding author at: Department of Physical Medicine and Rehabilitation, University of Michigan, 325 E. Eisenhower Ste. 100, Ann Arbor, MI 48109, United States. Tel.: +1 734 615 8543.

E-mail address: kristof@med.umich.edu (K. Kipp).

Applying FPCA to H-reflex recruitment curves may offer several benefits. First, this application would allow researchers to take advantage of FPCA's ability to analyze recruitment curves as a whole without loss of potentially important information. Second, even though a lot of data are entered into the analysis, the data reduction aspect of FPCA ensures that only the most essential information is retained (Ramsay and Silverman, 1997). Another benefit lies in the fact that the PCFs are extracted in a manner such that the first PCF captures the greatest amount of variance between recruitment curves, while the second PCF captures the second greatest amount of variance, and so forth (Ramsay and Silverman, 1997). Knowing the variances associated with each PCF would be practically significant, because researchers would be able to determine which characteristics account for the greatest variation in the data set and contribute most to differences between recruitment curves. Considering the benefits of FPCA, the primary purpose of this study was to use FPCA to analyze H-reflex recruitment curves.

2. Materials and methods

2.1. Participants

We recruited forty participants for a study that examined the relation between H-reflex measurements and neuromuscular performance. Data from thirty-eight participants (19 female and 19 male) of that study were used in this analysis. Two participants were excluded from testing because it was not possible to establish a full recruitment for these individuals. Participants were between 18 and 35 years of age and physically active a minimum of 30 min three times a week. Participants were free from current musculoskeletal injury, had not sustained any lower extremity injury within six months prior to participation, and had no history of lower extremity ligament surgery. In order to control for hormonal fluctuations across the menstrual cycle, females were tested on one of the three first days of their menstrual cycle.

Participants read and signed an informed consent form approved by the University's Institutional Review Board. A health history and training history questionnaire was used to verify eligibility for participation in the study. Leg dominance was determined via the preferred leg the participant: (a) kicked a ball, (b) recovered from a balance perturbation, and (c) stepped up on a 10 in. box. The leg used on the majority of the three tests was considered the dominant leg and was used for all subsequent testing. All reflex testing occurred in a seated semi-recumbent position. The knee was flexed to 60° and ankle in anatomical/neutral position (90° of plantar-dorsiflexion and 0° of inversion–eversion). The ankle was secured to a foot plate preventing any movement of the foot.

2.2. Electromyographical measurements

Lubricated Ag/AgCl surface EMG electrodes (Biopac Systems Inc, Goletta, CA, USA) were applied to the soleus muscle. Electrodes were placed longitudinally over the muscle belly with an inter-electrode distance of 2 cm. All EMG responses were collected and stored on a personal computer equipped with a Biopac MP100 data collection system (Biopac Systems Inc, Goletta, CA, USA). The EMG was sampled at 2000 Hz. To elicit the soleus H-reflex, a stimulating electrode (2 cm²) was placed over the tibial nerve in the popliteal fossa. A dispersal pad (3 cm²) was placed on the distal thigh just superior to the patella. H-reflex recruitment curves for the soleus were established by delivering a current to the tibial nerve in the popliteal fossa. Stimulation was delivered by a Grass S88 stimulator (Grass Technologies, West Warwick, RI, USA). A
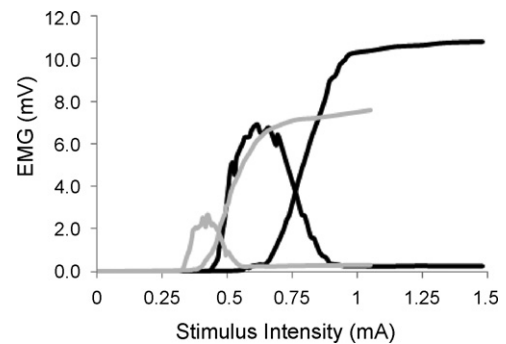

Fig. 1. Unprocessed H-reflex and M-wave recruitment curves from two participants (black and grey lines).

series of electrical stimuli (1 ms duration pulse), which progressively increased in intensity, were applied with a 10 s interstimulus latency period until the maximum motor response (M_{max}) was reached and plateaued (Fig. 1). The intensity of each stimulation was recorded and stored simultaneously with the EMG data. Each recruitment curve included between 50 and 75 stimulations.

2.3. Data analysis

H-reflex and M-wave recordings were made from the peak-to-peak amplitudes of each waveform at each stimulation intensity. However, only the recorded H-reflex portions of each recruitment curve were used for further analysis. All H-reflex recruitment curve data were then imported into a custom LabView (National Instruments Corporation, Austin, TX, USA) program that fitted each recruitment curve with a 4th order polynomial and linearly interpolated it to 100 data points. The 100th data point thus represents the stimulus intensity that elicited M_{max}. Furthermore, all points along each interpolated recruitment curve were normalized to M_{max}, such that the peak point of each curve represents the ratio between the maximum H-reflex and M_{max} (H_{max}/M_{max}). The slope of each recruitment curve was calculated by taking the first derivative of the recruitment curve. The maximum derivative was used to represent the peak slope (H_{slp}) of the ascending portion of the recruitment curve (Christie et al., 2004). The H-reflex threshold (H_{th}) was defined as the value where the recruitment curve exceeded 5% of the peak normalized reflex response (i.e., H_{max}/M_{max}).

For the FPCA the processed recruitment curves from all participants were combined into a single matrix **A** that consisted of 38 rows (participants) and 100 columns (interpolated data points) matrix (Ramsay and Silverman, 1997). In order to obtain the PCFs, the eigenvectors ξ of the covariance matrix **B** of the original data matrix **A** were extracted. The importance of each PCF is given by the eigenvalue for each eigenvalue–eigenvector pair, where the eigenvalues with the greatest absolute magnitude corresponds to the most prominent PCF that describes the greatest variance proportion in the data. The relative proportion of how much variance is explained in the data by each PCF is given by $\xi'\mathbf{B}\xi$. Only PCFs with eigenvalues greater than one and those that explained non-trivial variance (>3%) of the original data were retained for further analysis. The retained PCFs were then projected onto the original recruitment curve data $\xi'\mathbf{A}$. The summation of these projections gave a single score that determined how much of each PCF contributed to each participant's recruitment curve. An interpretation of the physical contribution of each PCF can be gained by reconstructing a recruitment curve, systematically increasing and decreasing the score of each PCF, and observing the subsequent effect on the reconstructed recruitment curve.

2.4. Statistical analysis

Standard linear correlation analyses were used to analyze associations between the discrete variables and the PCF scores of each PCF. The standard to show statistical significance was set an alpha-level of .05. All comparisons were made in SPSS (IBM Corporation, Somers, NY, USA).

3. Results

3.1. FPCA

The FPCA extracted three PCFs that cumulatively explained 93% of the variance in the H-reflex recruitment curves. The first PCF explained 56.0% of the variance, whereas the second and third PCFs explained 24.1% and 13.0% of the variance, respectively. Fig. 2 depicts the effects of systematically increasing and decreasing the score of each PCF. Changes in the score for the first PCF produced a primarily horizontal shift. Changes in the score of the second PCF produced a vertical shift and changes in the score of the third PCF changed the slope of the ascending portion of the recruitment curve.
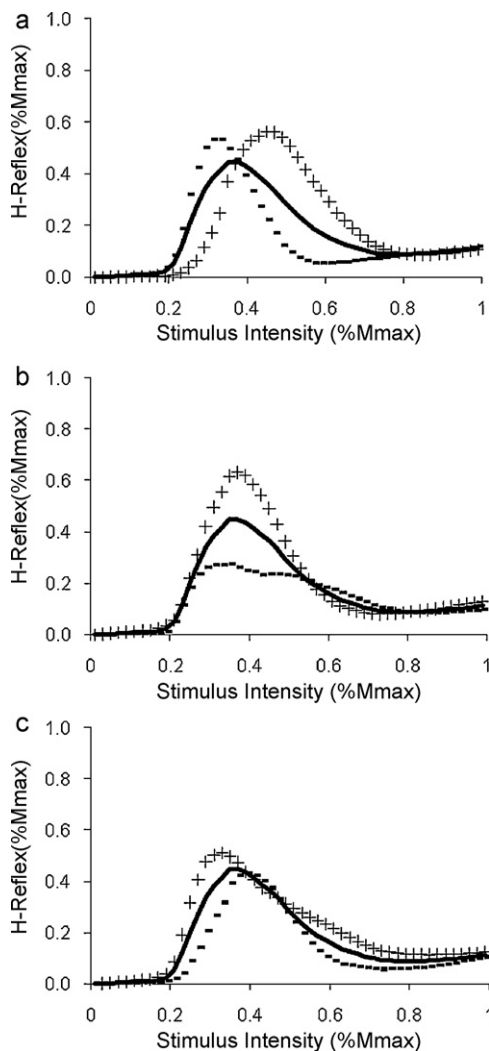

Fig. 2. Processed ensemble average H-reflex recruitment curves (solid black lines) for all 38 participants. Each sub-figure depicts the effects of systematically increasing ('+' symbols) or decreasing ('−' symbols) the scores for (a) the first principal component function, (b) the second principal component function, and (c) the third principal component function.

3.2. Correlations

The results of the correlation analysis are presented in Table 1. The scores for the first principal component function were significantly correlated with H_{max}/M_{max} ($p < .05$) and H_{th} ($p < .01$), while the scores for the second and third principal component functions were both significantly correlated to H_{max}/M_{max} and H_{slp} (all $p < .01$). Among the discrete variables, only H_{max}/M_{max} and H_{slp} were significantly correlated ($p < .01$). None of the principal component function scores were correlated.

4. Discussion

Our primary purpose was to use FPCA to analyze H-reflex recruitment curves. The FPCA extracted three PCFs and produced three sets of PCF scores, which were highly correlated to discrete variables derived from recruitment curves. In addition, systematic analysis of the FPCA results indicated that each PCF captured similar physiological characteristics as discrete variables. Furthermore, the FPCA provided variance proportions for each PCF and its physiological proxy, information not otherwise obtained from the analysis of discrete variables.

The FPCA extracted three principal component functions from the H-reflex recruitment curve data. Systematic variations to the PCF scores and each associated PCF allowed for a basic physiological interpretation of the FPCA results. Variation of the scores for the first PCF produced a primary horizontal shift in an individuals' recruitment curve, while changing the scores of the second PCF produced a vertical shift. Changing the scores for the third PCF changed the slope of the ascending portion of the recruitment curve. The results from the correlation analysis reflect these findings in that the scores of the first PCF are highly correlated to the H-reflex threshold (H_{th}). Likewise, the correlation analysis also indicated that the scores for the second and third PCFs are highly correlated to the H-reflex magnitude (H_{max}/M_{max}) and rate of excitability (H_{slp}). Considering that PCF scores were correlated to discrete variables, it appears that the FPCA derived PCFs are also able to capture aspects related to motoneuron pool excitability. This result suggests that FPCA provides a valid means to analyze H-reflex recruitment curves.

Among the discrete variables, the maximum H-reflex response (H_{max}/M_{max}) was significantly correlated to the peak slope of the recruitment curve (H_{slp}), which indicated that the rate of change in motoneuron excitability is in part related to the maximum H-reflex response. While this finding agrees with another report (Funase et al., 1994a), it also means that these variables share some variance and inevitably provide similar information about motoneuron pool excitability. Conversely, since the PCFs were not correlated at all, they capture unique and mutually exclusive characteristics of the recruitment curves. This finding illustrates a distinct benefit of using the FPCA approach in that the extracted data are orthogonal and not related to each other. The advantage of the lack of correlations between PCFs is that it ensures that the maximum amount of variation in recruitment curves is captured. Since the PCFs can capture more variation in the input data, they are also more sensitive to changes in experimental manipulation and more likely to locate significant effects than discrete variables (Chester, 2009; Ramsay and Silverman, 1997). Further, the lack of correlation between PCFs implies that the PCF scores could be used as variables in other statistical procedures without concern about multi-collinearity.

Another benefit of FPCA is that the PCFs are ordered according to the amount of variance they explain in the input data. Therefore, the first extracted PCF captures the greatest amount of variance between recruitment curves, while the second PCF captures the second greatest amount of variance, and so forth. The results from the current study indicated that the first PCF accounted for more

Table 1
Correlation matrix between discrete H-reflex measures (H_{max}/M_{max}, H_{slp}, H_{th}) and principal component function scores (PCF1, PCF2, and PCF3).

	Principal component functions			Discrete variables		
	PCF1	PCF2	PCF3	H_{max}/M_{max}	H_{slp}	H_{th}
PCF1	1					
PCF2	.000	1				
PCF3	.000	.000	1			
H_{max}/M_{max}	.334[*]	.623[**]	.577[**]	1		
H_{slp}	−.220	.435[**]	.456[**]	.655[**]	1	
H_{th}	.712[**]	−.219	−.149	.146	−.088	1

[*] $p < .05$.
[**] $p < .01$.

than half of the variation in all recruitment curves, the second PCF accounted for about a quarter, and the third PCF accounted for roughly 13%. Knowledge of the variance distributions for a given set of PCFs is of great practical significance, because it allows researchers to determine either the relative importance of each PCF or the amount variation that is associated with it. Combining this information with the physiological interpretation from the correlation analysis and the systematic variation in PCF scores would therefore indicate that the dominant source for variations in the recruitment curves of the subjects in the current study was a horizontal shift in recruitment curves that was related to the H-reflex threshold (H_{th}). In comparison, the PCFs related to H-reflex magnitude (H_{max}/M_{max}) and rate of excitability (H_{slp}) accounted for relatively little variation between recruitment curves of all subjects. Furthermore, a potentially useful extension of the FPCA approach would be to examine how the variance proportions change in response to an intervention. Essentially, a within-subject analysis could be used to study how PCFs and PCF scores change in response to acute perturbations or chronic influences. For example, using a FPCA to analyze recruitment curves before and after an exercise protocol (e.g., strength training) would provide insight into how various physiological aspects captured by PCFs change and provide information of how measures of motoneuron pool excitability are influenced by such interventions.

While the FPCA approach in the analysis of recruitment curves has several benefits, there are also a few issues related to post data collection processing, such as smoothing and interpolation, which warrant discussion. These issues arise from potential methodological problems associated with eliciting H-reflexes and establishing recruitment curves. First, the necessary number of stimulations needed to establish recruitment curves may vary between individuals. The FPCA, however, necessitates that all input data are of equal length (i.e., have the same amount of data points). Interpolation of recruitment curves is therefore necessary to ensure that FPCA can be performed. In addition, inter-individual differences may also lead to unequal intervals between stimulation intensities. This, however, poses a problem when the data need to be differentiated, which is necessary to calculate the slope of each curve. Considering these methodological issues, the need for data processing thus becomes apparent. Previous research, however, suggests that discrete variables derived from similarly processed recruitment curves still yield reliable measures (Christie et al., 2004). Potential compromises for these issues may be achieved if only the ascending portion of the recruitment curve were to be analyzed or if several recordings were made at each stimulation intensity (Capaday, 1997; Funase and Miles, 1999).

This study used FPCA to analyze H-reflex recruitment curves. FPCA decomposed recruitment curves and extracted a smaller set of PCFs that were correlated to discrete measures of motoneuron pool excitability and were ordered by the variance they explained, but were not correlated to each another. The application of FPCA in the analysis of H-reflex recruitment curves should be considered for use in future studies that investigate excitability of the motoneuron pool. A particular fruitful use may be found in using this method for within-subject paradigms to study the changes in variance proportions of measures associated with motoneuron pool excitability in response to acute perturbations or chronic influences.

References

Capaday C. Neurophysiological methods for studies of the motor system in freely moving human subjects. J Neurosci Methods 1997;74:201–18.

Chester V. Using waveform analyses to develop pediatric gait indices. Exerc Sport Sci Rev 2009;37:211–7.

Christie A, Lester S, LaPierre D, Gabriel DA. Reliability of a new measure of H-reflex excitability. Clin Neurophysiol 2004;115:116–23.

Daffertshofer A, Lamoth CJ, Meijer OG, Beek PJ. PCA in studying coordination and variability: a tutorial. Clin Biomech 2004;19:415–28.

Funase K, Imanaka K, Nishihira Y. Excitability of the soleus motoneuron pool revealed by the developmental slope of the H-reflex as reflex gain. Electromyogr Clin Neurophysiol 1994a;34:477–89.

Funase K, Imanaka K, Nishihira Y, Araki H. Threshold of the soleus muscle H-reflex is less sensitive to the change in excitability of the motoneuron pool during plantarflexion or dorsiflexion in humans. Eur J Appl Physiol 1994b;69:21–5.

Funase K, Miles TS. Observations on the variability of the H reflex in human soleus. Muscle Nerve 1999;22:341–6.

Hayes BT, Hicks-Little CA, Harter RA, Widrick JJ, Hoffman MA. Intersession reliability of Hoffmann reflex gain and presynaptic inhibition in the human soleus muscle. Arch Phys Med Rehabil 2009;90:2131–4.

Knikou M, Angeli CA, Ferreira CK, Harkema SJ. Soleus H-reflex gain, threshold, and amplitude as function of body posture and load in spinal cord intact and injured subjects. Int J Neurosci 2009;119:2056–73.

Nielsen JB. Sensorimotor integration at spinal level as a basis for muscle coordination during voluntary movement in humans. J Appl Physiol 2004;96:1961–7.

Ramsay JO, Silverman BW. Functional data analysis. New York: Springer; 1997.

Stein RB, Thompson AK. Muscle reflexes in motion: how, what, and why? Exerc Sport Sci Rev 2006;34:145–53.

Tucker KJ, Tuncer M, Turker KS. A review of the H-reflex and M-wave in the human triceps surae. Hum Mov Sci 2005;24:667–88.

Zehr PE. Considerations for use of the Hoffmann reflex in exercise studies. Eur J Appl Physiol 2002;86:455–68.