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RESEARCH ARTICLE

A sigmoid function is the best fit for the ascending limb of the Hoffmann reflex recruitment curve

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Abstract The Hoffmann (*H*)-reflex has been studied extensively as a measure of spinal excitability. Often, researchers compare the *H*-reflex between experimental conditions with values determined from a recruitment curve (RC). An RC is obtained experimentally by varying the stimulus intensity to a nerve and recording the peak-to-peak amplitudes of the evoked *H*-reflex and direct motor (*M*)-wave. The values taken from an RC may provide different information with respect to a change in reflex excitability. Therefore, it is important to obtain a number of RC parameters for comparison. RCs can be obtained with a measure of current (HCRC) or without current (HMRC). The ascending limb of the RC is then fit with a mathematical analysis technique in order to determine parameters of interest such as the threshold of activation and the slope of the function. The purpose of this study was to determine an unbiased estimate of the specific parameters of interest in an RC through mathematical analysis. We hypothesized that a standardized analysis technique could be used to ascertain important points on an RC, regardless of data presentation methodology (HCRC or HMRC). For both HCRC and HMRC produced using 40 randomly delivered stimuli, six different methods of mathematical analysis [linear regression, polynomial, smoothing spline, general least squares model with custom logistic (sigmoid) equation, power, and logarithmic] were compared using goodness of fit statistics (*r*-square, RMSE). Behaviour and robustness of selected curve fits were examined in various applications including RCs generated during movement and somatosensory conditioning from published data. Results show that a sigmoid function is the most reliable estimate of the ascending limb of an *H*-reflex recruitment curve for both HCRC and HMRC. Further, the parameters of interest change differentially with respect to the presentation methodology and the analysis technique. In conclusion, the sigmoid function is a reliable analysis technique which mimics the physiologically based prediction of the input/output relation of the ascending limb of the recruitment curve. Therefore, the sigmoid function should be considered an acceptable and preferable analytical tool for *H*-reflex recruitment curves obtained with reference to stimulation current or *M*-wave amplitude.

Keywords *H*-reflex · Methodology · Recruitment curve · Motor control

Introduction

The Hoffmann (*H*)-reflex has been used extensively to measure changes in spinal excitability in motor control experiments (Misiaszek 2003; Pierrot-Deseilligny et al. 2005; Zehr 2002). However, evoking and analyzing the

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H-reflex requires awareness of many physiological and technical assumptions (Misiaszek 2003; Zehr 2002). Often, an *H*-reflex recruitment curve is used to compare the reflex response at various levels of stimulus intensity. The methodology of a recruitment curve involves varying the level of electrical stimulation delivered to a mixed nerve and measuring the peak-to-peak amplitudes of the two resulting waveforms in the electromyogram. The direct motor wave (*M*-wave) and the *H*-reflex wave (*H*-wave) are compound muscle action potentials that result from the stimulation of motor and sensory fibres, respectively. The responses of these waveforms to changes in intensity of stimulation define the input/output relation of each phenomenon which is highlighted by specific parameters of interest that can be used for experimental comparison.

Specifically, the ascending limb of the *H*-reflex RC is isolated, and parameters such as the threshold of the response (H_{TH}), the maximum reflex response (H_{MAX}) and the slope of the ascending limb (H_{SLP}) are used to represent alterations in the *H*-reflex input/output function. Experimental protocols may differentially affect one or all of these parameters, and therefore it is often beneficial to acquire many measures of *H*-reflex excitability. Currently, there are two accepted methods of presentation of the *H*-reflex recruitment curve. The first is to present both the *H*-reflex amplitudes and *M*-wave amplitudes versus stimulus amplitude (current or integrated current) measured using a current monitor. This is the suggested method of presentation if a current monitor is available. The developed plots are called *H*-reflex/current recruitment curve (HCRC) and *M*-wave/current recruitment curve (MCRC). However, if a current monitor is not available an alternative method of presentation is to plot the *H*-waves as a function of the size of the concurrently evoked *M*-wave, and the developed plot is called an *H*-wave/*M*-wave recruitment curve (HMRC). There has yet to be a comparison between presentation methodologies with respect to changes in parameters of interest. It is therefore important to evaluate the results of an experiment using both presentation methodologies to determine how changes in the input/output relation of HCRC and HMRC compare.

The determination of a function that can approximate the ascending limb of the recruitment curve requires great physiological and methodological consideration. It has been assumed that the ascending limb follows a linear response (Funase et al. 1994a, 1996). It is for this reason that Funase et al. (1994a) chose to use linear regression to analyse the ascending limb of the *H*-reflex recruitment curve. However, the rate of recruitment of alpha motor neurons may follow an exponential function (Fuglevand et al. 1993; Jones 2005). Therefore, it is necessary to determine a mathematical analysis technique to properly approximate the experimental data. The use of linear regression to analyse the ascending limb of the recruitment curve is a currently accepted method. However, linear regression has never been tested against other mathematical analysis techniques such as a recently presented polynomial technique (Christie et al. 2004) to determine its validity.

In the first part of this study we compared six mathematical analysis techniques of the ascending limb of the *H*-reflex recruitment curve for both HCRC and HMRC with measures of goodness of fit. We hypothesized that the most suitable analysis technique should conform to both physiological and technical assumptions, perform well on measures of goodness of fit as well as produce parameters of interest used for experimental comparison. This part of the study included a comparison of manual versus automated methods of determining the ascending limb data set for selected curve fitting techniques. The second part of the study involved the quantification of changes in parameters of interest from two mathematical analysis techniques (linear and sigmoid) during movement and somatosensory conditioning experiments for both HCRC and HMRC. Through this comparison we show that changes in the parameters of interest represent important considerations that are defined by the mathematical analysis technique and presentation methodology.

Methods

Participants

For part I of the experiment three participants (age range 29–42 years) with no known peripheral or central neurological conditions volunteered. In part II previously published data were analysed using linear and sigmoid analysis techniques for both HCRC and HMRC presentation methodologies (Zehr et al. 2007a). These data are used solely to present differences in analysis techniques and presentation methodologies and do not contradict or embellish currently published physiological results. All participants gave written consent to a protocol approved under the Human Ethics and research Committee at the University of Victoria and performed in accordance with the Declaration of Helsinki.

EMG

EMG was recorded using Ag–AgCl bipolar configurations of surface electrodes (Thought Technologies Ltd., Montreal, QC, Canada) from the left and right Soleus (SOL) and Tibialis Anterior (TA) muscles for experiment I. The area over the muscle sites was cleaned with rubbing alcohol prior to application. Individual ground electrodes were placed over bony landmarks near each muscle.

Soleus *H*-reflexes

The left and right (posterior) tibial nerves were stimulated at the popliteal fossa using 1-ms square wave pulses to evoke the *H*-reflex using bipolar surface electrodes and a Digitimer (Medtel, NSW, Australia) constant current stimulator (model DS7A). Nerve stimulation was delivered pseudo randomly between 3 and 5 s apart during all trials. Current was measured using an mA-2000 Noncontact Milliammeter (Bell Technologies, Orlando, FL, USA). For part I two *H*-current recruitment curves ($n = 40$ sweeps) were constructed for each leg for a total of four recruitment curves per subject. Also, 10 electrically evoked *H*-waves were recorded at each of five stimulus intensities chosen from the ascending limb of all recruitment curves. All recordings were taken while the subjects were at rest. For part II full *H*-reflex recruitment curves ($n = 40$ sweeps) were obtained in all conditions. Control recruitment curves were also constructed at the beginning and at the end of each experiment. In the somatosensory conditioning experiment soleus *H*-reflexes were conditioned with Superficial Radial (SR) nerve stimulation. SR nerve stimulation was delivered using trains of 5 × 1.0 ms pulses at 300 Hz with a condition-test (C-T) interval of 100 ms (Zehr et al. 2001). Electrodes for cutaneous nerve stimulation were placed on the dorsal surface of the forearm just distal to the radial head in the anatomical snuff box. Stimulus intensity was set at twice the threshold at which a clear radiating paresthesia was reported (Haridas and Zehr 2003; Zehr et al. 2001).

Protocol

For all static control trials, subjects were seated with knees bent at an ~90° angle and instructed to maintain the same posture throughout the experiment. For part I the participant's soleus muscle was relaxed for all trials, whereas part II required subjects to maintain a low level of contraction (~20 MVC) in the soleus muscle ipsilateral to the site of stimulation. In part I *H*-reflexes were evoked in both the left and right legs, whereas only the right leg was stimulated in part II. Also, in part II participants wore an ankle-foot orthosis (AFO) on their right side and were provided visual feedback of soleus contraction level on an analogue oscilloscope. The protocol for part II was similar to previous experiments involving the effect of leg and arm cycling on reflex modulation (Balter and Zehr 2007). Subjects performed two movement tasks at a frequency of 1 Hz: (1) arm cycling with legs stationary with knees bent at an ~90° angle (ARM); (2) leg cycling with arms stationary (LEG). Also, *H*-reflexes were evoked while participants performed static postures matching the two cycling tasks to provide control conditions for each task. As described previously (Balter and Zehr 2007), an arm and leg cycle ergometer (PRO II, SCIFIT Systems Inc., Tulsa, OK, USA) was used. Reflexes were evoked at the late leg extension power phase (~1–3 o'clock position) of the movement as an optical encoder monitored the position of the arm and leg cranks.

Data acquisition and analysis

Data were acquired at a sampling rate of 5,000 Hz with a 12-bit A/D converter connected to a computer running custom-written (Dr. Timothy Carroll, University of New South Wales, Australia) Lab View software (National Instruments, Austin, TX, USA). TA, AD and VL EMG signals were preamplifed with a gain of 5,000, band pass filtered at 100–300 Hz (P511 Grass Instruments, AstroMed Inc., Westwarwick, RI, USA) and full-wave rectified. Soleus EMG was preamplifed with a gain of 500 and band pass filtered at 100–1,000 Hz. Soleus *H*-reflex EMG data were analysed using single, unrectified sweeps. *H*-reflex peak-to-peak amplitudes were analysed in all trials. For each subject *M*-waves and *H*-reflexes were normalized to the corresponding M_{MAX} to reduce inter-subject variability. For part I, HCRC and HMRC recruitment curves (ascending limb only) were fit with mathematical analysis techniques outlined below and compared with respect to measures of goodness of fit. For part II, the ascending limbs of recruitment curves were fit using linear regression and a sigmoid function for both HCRC and HMRC. All parameters of interest and estimated values (outlined below) were used to compare between analysis technique and presentation methodology.

Comparison of mathematical analysis techniques

A custom-built analysis program was designed (National Instruments Labview 8^{TM}, Austin, TX, USA) to compare the same data set using different analysis techniques. The techniques compared were linear regression, polynomial, smoothing spline, power, logarithmic, and a general least squares fit to a custom logistic equation (sigmoid). The data were prepared for all fitting techniques by centring and scaling the current data to improve the fit accuracy and decrease the computational complexity of the procedures. Centring was achieved by subtracting the mean to centre the data on a zero point, and scaling involved dividing the data by the standard deviation. After each fitting procedure, the current values were re-centred and re-scaled to properly align the data and the resultant curve fits. The amplitudes of the *H*-reflex and *M*-wave responses were normalized to the average maximum *M*-wave (M_{MAX}) obtained from each RC (Crone et al. 1999; Frigon et al. 2007). The current values for all fits were normalized to the current at 50% of M_{MAX} obtained from a sigmoid fit to the MCRC. M_{MAX} was determined for all RC as the mean of the five maximum *M*-wave values. All equations and descriptions of fitting methods can be obtained from The National Instruments^{TM} website (http://zone.ni.com/reference/en-XX/help/371361B-01/gmath/curve_fitting_vis/). A brief description of the analysis techniques is provided in the Appendix.

Methods of determining the peak of the ascending limb

The method for determining the ascending limb of the curve fit was evaluated using an automated and a manual method. The methods evaluated for setting the upper limit of the ascending limb of the recruitment curve were calculated (calc), chosen (chos), polynomial (poly) and smoothing spline (ss). These methods defined the boundaries of the ascending limb. In the calc method, the computer program defined the peak of the ascending limb as the data point with the largest magnitude. The chos technique involved the manual selection of the upper limits of the ascending limb. The poly technique defines the peak of the ascending limb as the value corresponding to the maximum amplitude of a 9th order polynomial to the given set of data. The ss technique defines the ascending limb as all values below the current value corresponding to the maximum amplitude of a smoothing spline curve fit using the error fitting technique (see Appendix).

Experimental parameters of interest

There were six parameters of interest taken from the curve fits that could be used for experimental evaluation of changes in the HRC (Fig. 1). These values are H_{MAX}, 50% H_{MAX}, H_{SLP}, current at H_{TH}, current at 50% H_{MAX} and current at H_{MAX}.

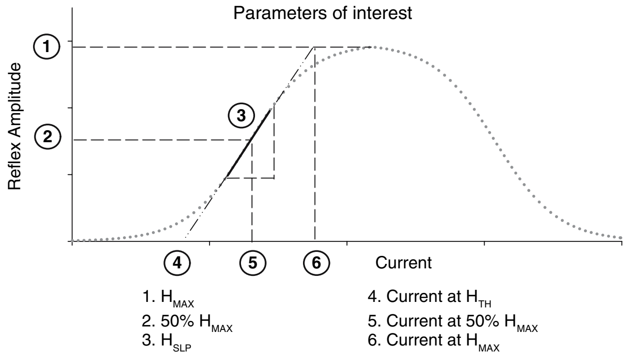


Fig. 1 *H*-reflex recruitment curve parameters of interest. Specific parameters are taken from the ascending limb of the HRC for experimental comparison. These parameters are: *1* H_{MAX}—the maximum amplitude of the reflex response. *2* 50% H_{MAX}—half of the maximum reflex amplitude response. *3* H_{SLP}—the slope of the ascending limb. *4* Current at H_{TH}—the current value associated with the first noticeable reflex response. *5* Current at 50% H_{MAX}—the current value associated with the reflex response at 50% H_{MAX}. *6* Current at H_{MAX}—the current value associated with the maximum amplitude reflex response

H_{MAX} was defined as the average of the five largest *H*-reflex amplitudes for the linear, power, logarithmic, and sigmoid curve fits. For the smoothing spline and polynomial curve fits H_{MAX} was defined as the maximum of the generated function. 50% H_{MAX} was defined from all curve fits as one-half of the H_{MAX} value. H_{SLP} was defined as the slope (m) from the linear fit. For power, logarithmic, smoothing spline and polynomial H_{SLP} was defined as the derivative of the function at 50%H_{MAX}. For the sigmoid curve fit H_{SLP} was defined as the slope of the ascending limb of the recruitment curve at 50% of the H_{MAX} value. This slope was determined using Eq. 1:

$$\frac{m(H_{MAX})}{4} \tag{1}$$

where H_{MAX} is the upper limit of the curve and m is the slope parameter of the function. The current at 50%H_{MAX} from linear, smoothing spline, polynomial, power and logarithmic fits was determined as the current value corresponding to the 50%H_{MAX} value of the function. The current at 50%H_{MAX} for the sigmoid function is a direct output parameter (*s50*-see Appendix). The current at H_{TH} from the linear fit corresponds to the x-intercept of the linear function. For the remaining curve fits current at H_{TH} was defined as the x-intercept of the linear functions developed using the H_{SLP} and current at 50% H_{MAX} values obtained from those curve fits (Fig. 1). The necessity of this method is due to the rapid increasing, decreasing (sigmoid, power, logarithmic) or erratic (smoothing spline, polynomial) nature of the curve fits near the limits of the data. This is similar to a procedure presented elsewhere when evaluating the motor evoked potential (MEP) input/output relation (Devanne et al. 1997) and recently the *H*-reflex recruitment curve (Zehr et al. 2007a; Zehr et al. 2007b). Current at H_{MAX} for the linear fit was defined as the current value obtained from the function with H_{MAX} as an input. Current at H_{MAX} for smoothing spline, polynomial, power, logarithmic and sigmoid was defined as the intersection of the linear function created from the H_{SLP}, and current at 50% H_{MAX} for each function with the H_{MAX} determined from each curve fit function.

Predicted values

The variables of H_{MAX}, 50% H_{MAX}, H_{TH} taken from the static control curves were compared to those from the same currents values on the conditioned curves (Fig. 2) (Zehr and Klimstra 2006).

That is, the same relative current needed to evoke a certain sized *H*-reflex on the static control recruitment curve was input into the movement curve fit to obtain an estimated value. To differentiate the description of reflex parameters taken from the fitted curves, they are described as "@" the value from static control. For example, modulation of the value for H_{MAX} during static is $H_{@\mathrm{MAX}}$ during cycling tasks. This is similar in principle to a procedure applied using linear fits (Zehr and Stein 1999).

Goodness of fit statistics

RMSE

The root mean square error is a measure of the amplitude difference between the values obtained from the curve fit and the sampled data. The difference can occur due to variability in the data or because the curve fit does not account for variables that could result in a more accurate fit.

R-*square*

R-square is a measure of the percentage of variability in the original data that is accounted for by the fitted curve. Because the *r*-square value does not exist on a linear scale all *r*-square data were converted to *r*-values and then underwent a Fischer *z*-transform to allow the measures of *r* between curve fits to be properly compared.

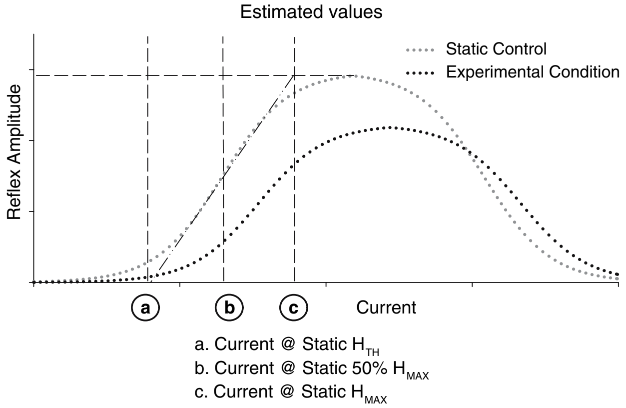


Fig. 2 Estimated values taken from *H*-reflex recruitment curve fits compared across experimental conditions. The current values associated with the static control RC are input into the curve fit of another experiment condition to produce estimated values of *H*-reflex response at the same current intensities

Statistics

STATISTICA software (StatSoft, Tulsa, OK, USA) was used to perform repeated measures analyses of variance (ANOVA) with Tukey's HSD post hoc and Student's *t*-tests. Descriptive statistics included means ± standard error of the mean (*SEM*). Statistical significance was set at $P \leq 0.05$. For part I separate analyses were conducted for measures of goodness of fit (*r*-square, RMSE). Where ANOVA results revealed significant main effects Tukey's *HSD post hoc* tests were used to identify the specific difference. Student's *t*-tests were used to examine differences between the curve fits using a manually chosen ascending limb and those with automated choices. For Part II repeated measures ANOVA were performed separately for the parameters of interest and predicted values for each analysis type and presentation methodology. Where ANOVA results revealed significant main effects Tukey's *HSD post hoc* tests were used to identify the specific difference

Results I: comparison of mathematical analysis techniques

Analysis technique comparison

Figure 3 shows single subject HCRC and HMRC data with all curve fits. Note that the fitting techniques were limited in the HMRC to linear, polynomial and sigmoid. These were the only techniques available for this comparison because the smoothing spline, power and logarithmic fits were unable to fit data with repeated data points. That is, where few data were sampled with different evoked amplitudes at the same *M*-wave amplitude. Five amplitude values from each developed curve were compared to the five averaged *H*-amplitudes at their respective current or *M*-wave values through an analysis of Fisher *z*-transformed *r*-values and RMSE. For the HCRC comparison statistical analysis revealed significant differences between the *r*-values of the smoothing spline, polynomial, sigmoid and logarithmic fits from the linear and power fits yielding significantly higher values of *r* ($P < 0.05$). Figure 4a, b shows the Fisher *z*-transformed *r*-values for all curve fits as compared to the five averaged values for both HCRC and HMRC.

Figure 4c, d shows the RMSE values for all curve fits. The sigmoid, polynomial, smoothing spline and logarithmic fits produced the smallest values of RMSE. These

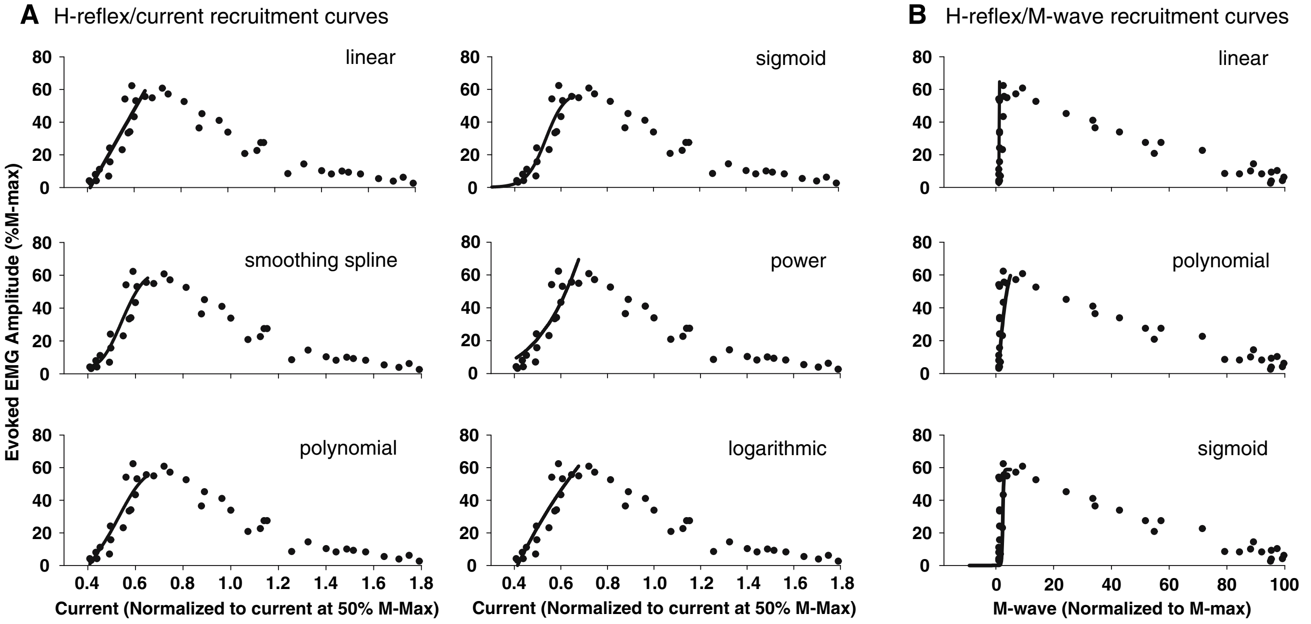
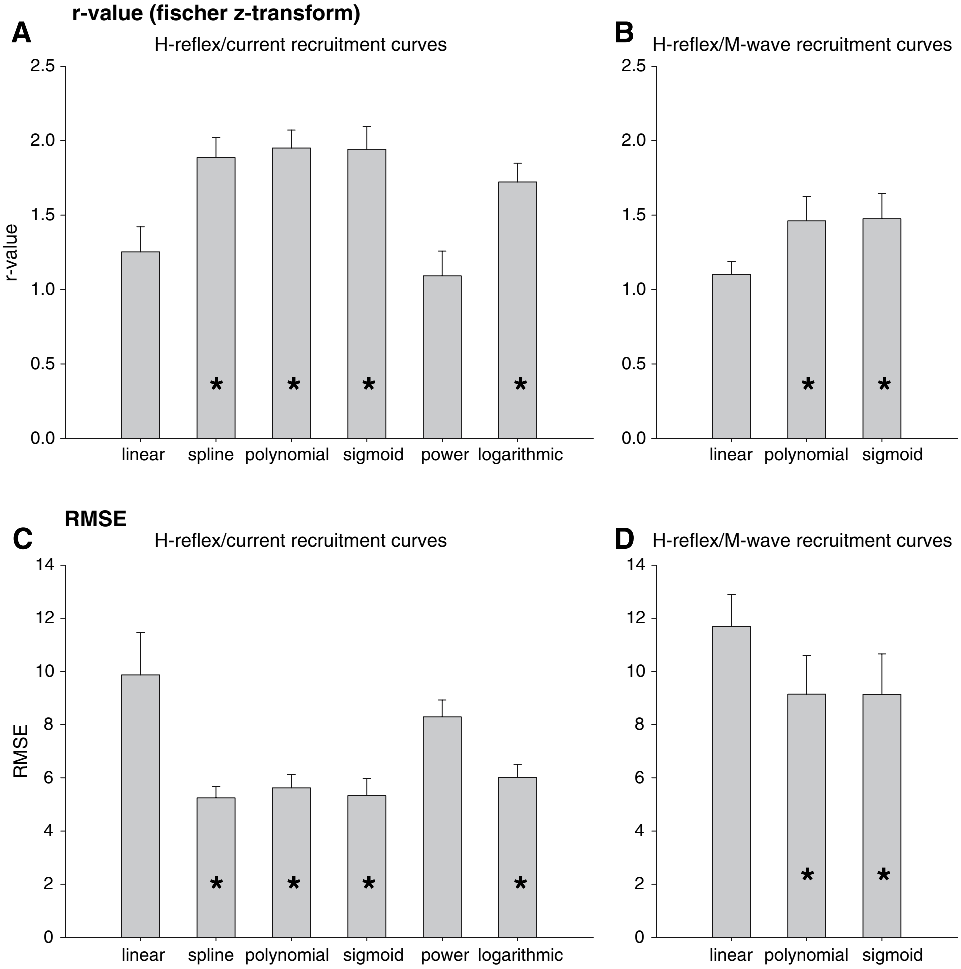


Fig. 3 Different curve fitting procedures displayed on HCRC and HMRC. **a** All fitting procedures for HCRC compared in the current experiment (linear, power, logarithmic, smoothing spline, polynomial, sigmoid) displayed for a single subject. **b** All fitting procedures for HMRC compared in the current experiment (linear, polynomial, sigmoid) displayed for a single subject

Fig. 4 Goodness of Fit statistical results. **a** The fisher transformed *r*-values for all curve fits in HCRC. **b** The fisher transformed **r**-values for all curve fits in HMRC. **c** The RMSE from all curve fits in HCRC. **d** The RMSE from all curve fits in HMRC. For HCRC, smoothing spline, polynomial, sigmoid and logarithmic are better fits to the experimental data than the linear and power fits as evaluated by *r*-value and RMSE. For HMRC, polynomial and sigmoid are better fits to the experimental data than the linear fit as evaluated by *r*-value and RMSE. All data are means ± SEM



values were found to be significantly better fits than the power and linear fits. For the HMRC analyses, statistical analysis showed that the polynomial and sigmoid fits produced significantly larger *r*-values and significantly smaller values of RMSE than the linear fit. There was no significant difference between any of the groups when comparing the manual versus automated techniques suggesting that any of these methods may produce equivalent results.

Results II—movement conditioning

The purpose of this section is to show the results of a physiological experiment evaluated using different analysis techniques and presentation methodologies. This comparison does not intend to expand upon or contradict the presentation of data already published in a research report (Zehr et al. 2007a).

Movement conditioning induces modulation of *H*-reflex RC (Zehr et al. 2007a). The conditions compared were static (no movement), arm cycling and leg cycling. Statistically significant differences noticed between movement conditions are ranked numerically with respect to the occurrence of a significant difference shown in all parameters of interest. This ranking allows a comparison of the sensitivity of analysis techniques and will be used as a gauge of the suitability of each analysis technique and presentation methodology, where level 1 is the least sensitive comparison and level 3 is the most sensitive. Single subject recruitment curves during static and movement conditions are presented in Fig. 5. All significant comparisons of parameters of interest and estimated data between experimental interventions are presented in Table 1.

Table 1 presents the significant differences noticed between movement conditions for both linear and sigmoid analysis techniques for HCRC and HMRC data presentation methodologies. As can be seen from Table 1 for all analysis techniques and presentation methodology analysis of H_{MAX} and H_{SLP} showed consistent results. That is, a significant difference was noticed between all levels of movement conditions for H_{MAX}. This is an expected result because the method for determining H_{MAX} is identical for all analysis techniques and presentation methodologies. For the analysis of H_{SLP} all analysis techniques and presentation methodologies showed the same sensitivity between experimental conditions only showing a difference between levels 1 and 2. For current (*M*-value) at threshold, HCRC sigmoid showed the greatest sensitivity with differences between all levels of movement comparison, whereas all other analysis techniques and presentation methodologies failed to show any difference. For the current (*M*-value) at 50%H_{MAX} the HCRC sigmoid showed sensitivity for movement comparison at all three levels, whereas HMRC linear and sigmoid showed differences at levels 1 and 2, and HCRC linear failed to show any differences. For current (*M*-value) at H_{MAX} HCRC and HMRC sigmoid were able to show differences between levels 1 and 2, whereas HMRC linear only showed differences at level 1 and HCRC linear showed no differences between conditions. For the $H_{@\mathrm{TH}}$ predicted value the HCRC sigmoid was sensitive to all levels of

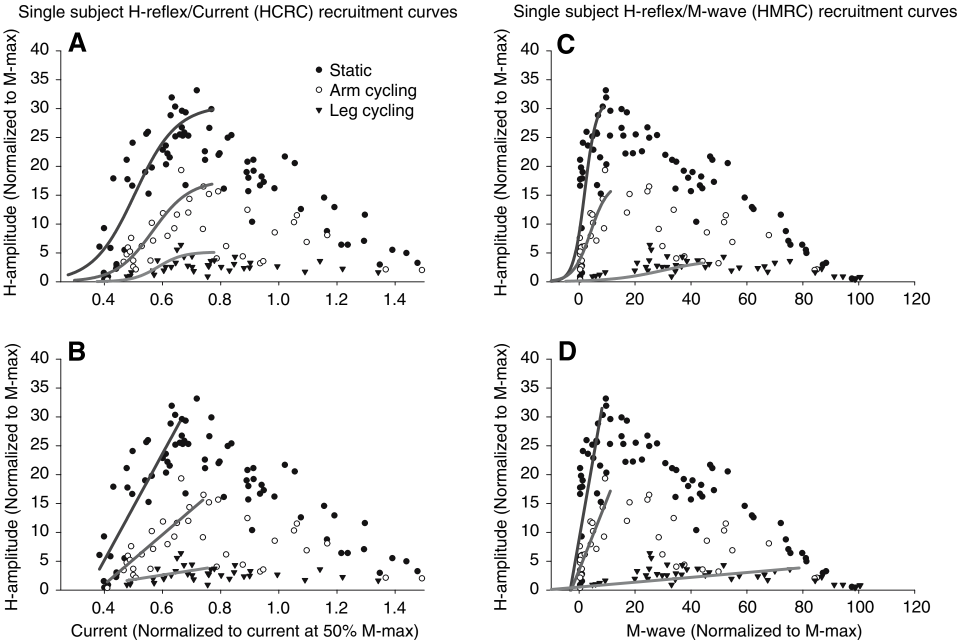


Fig. 5 Single subject HCRC and HMRC during movement conditioning fit with a sigmoid fitting technique (**a**, **c**) and a linear fitting technique (**b**, **d**)

Table 1 Statistical differences found during movement conditioning for different analysis techniques and presentation methodologies

	HCRC		HMRC	
	Linear	Sigmoid	Linear	Sigmoid
Parameters of interest				
H_{MAX}	123	123	123	123
H_{SLP}	12	12	12	12
Current (M-value) at threshold		123		
Current (M-value) at 50% H_{MAX}		123	12	12
Current (M-value) at H_{MAX}		12	1	12
Estimated values				
$H_{@\mathrm{TH}}$	2	123		12
$H_{@50\%\mathrm{MAX}}$	123	123	12	123
$H_{@\mathrm{MAX}}$	12	123	12	123
Total 1 (percentage)	50	100	75	82.5
Total 2 (percentage)	62.5	100	62.5	82.5
Total 3 (percentage)	25	75	12.5	37.5

The parameters of interest and estimated values (rows) are compared between presentation methodologies and analysis techniques (columns). Movement Conditions are ranked from 1 to 3 in order of the occurrence of a significant difference noticed in all presentation methodologies and analysis techniques. The three bottom rows show the cumulative percentage of the three ranked differences for each presentation methodology and analysis technique

1 static significantly different than legs

2 arms significantly different than legs

3 static significantly different than arms

movement conditioning, the HMRC sigmoid was sensitive to levels 1 and 2, HCRC linear was sensitive only to level 2 and HMRC linear was not sensitive at any level. At $H_{@50\%\mathrm{MAX}}$ HCRC sigmoid and linear as well as HMRC sigmoid showed differences between all levels of movement conditioning, and HMRC linear showed differences between levels 1 and 2. For $H_{@\mathrm{MAX}}$ HCRC sigmoid and HMRC sigmoid showed significant differences between all levels of movement conditioning, whereas HCRC linear and HMRC linear were only sensitive to levels 1 and 2.

Results II—somatosensory conditioning

The results from this section are presented to show important differences between analysis techniques and presentation methodologies for the determination and evaluation of certain parameters of interest. Single subject recruitment curves during static and somatosensory conditioning are presented in Fig. 6.

For all analysis type and presentation methodologies there were no differences between conditions for H_{MAX}. An important finding is that the parameter of H_{SLP} showed significant differences only for the HMRC analysis techniques. For current (M-value) at threshold there were no differences between conditions for any analysis technique or presentation methodology. HCRC linear and sigmoid showed significant differences between conditioned and unconditioned reflexes for current at 50%H_{MAX} and current at H_{MAX}, whereas the HMRC analysis techniques showed no difference between conditions. For $H_{@\mathrm{TH}}$ a significant difference was noticed only for the HCRC sigmoid fit. For $H_{@50\%\mathrm{MAX}}$ significant differences were noticed for all HCRC and HMRC linear and sigmoid. For $H_{@\mathrm{MAX}}$ significant differences were noticed for only for HCRC and HMRC linear fits.

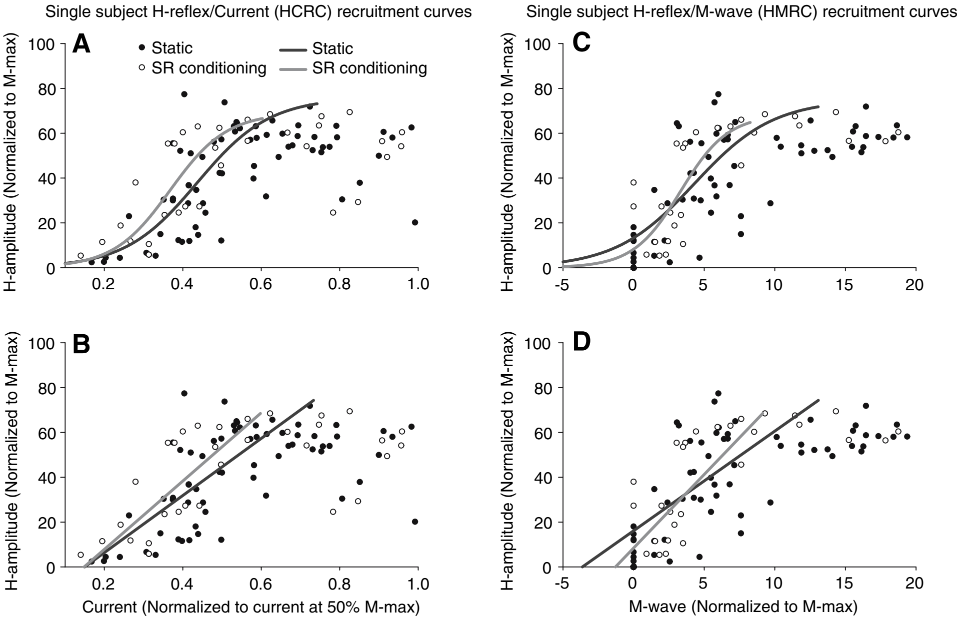


Fig. 6 Single subject HCRC and HMRC during somatosensory conditioning fit with a sigmoid fitting technique (**a**, **c**) and a linear fitting technique (**b**, **d**). Notice how the different presentation methodologies (HCRC and HMRC) correspond to differential changes in the parameters of interest evident in both sigmoid and linear curve fits

Discussion

The main finding from the comparison of analysis techniques shows that the smoothing spline, polynomial, sigmoid and logarithmic curve fits are better fits to the experimental data than the power fit and the accepted linear regression technique through a measure of goodness of fit statistics (*r*-square, RMSE). This would suggest that both the HCRC and HMRC follow a non-linear response to increasing stimulus intensity, and therefore analysis techniques that allow a more dynamic evaluation of the input/output function should be considered. Certain considerations for the choice of a valid analysis technique include both the physiological justification of a chosen technique and the ability of the technique to obtain parameters of interest for experimental comparison.

Physiological and methodological justification for a sigmoid fit

Many researchers have described the ascending limb of the *H*-reflex RC as sigmoidal (Christie et al. 2004; Hoehler and Buerger 1981; Slot and Sinkjaer 1994; Stein et al. 2007; Wilmink et al. 1996). Accordingly, there is sufficient evidence to consider that the ascending limb of the HCRC and HMRC follows a sigmoid function. To begin with, there is assumed to be an exponential distribution of recruitment thresholds across the motorneuron pool (Fuglevand et al. 1993; Jones 2005; K. Jones, personal communication). This results from a large proportion of low threshold motor units with exponentially diminishing number of high threshold units (Fuglevand et al. 1993). Therefore, as the stimulus to elicit an *H*-reflex increased there would be an exponential response noticed at the foot of the curve. Also, as evoked stimulus elicits a response along the motor axons, antidromic volleys would collide with orthodromic volleys, thus blunting the exponential rise and approximating a logarithmic decrease in the ascending limb near the peak of the curve (Funase et al. 1994b; Pierrot-Deseilligny et al. 2005; Stein et al. 2007).

A methodological justification for the use of a sigmoid function to approximate the ascending limb of the *H*-reflex RC is also viable. The variability in the response characteristics suggests that even if the ascending limb of the *H*-reflex recruitment curve is a pure linear response, the variability is not equivalent at all levels of stimulus intensity. That is, the variability of the *H*-reflex would be susceptible to a floor effect and a ceiling effect at the foot and the peak of the ascending limb, respectively. Thus, the sampled data from the foot of the curve and the peak of the curve would overestimate and underestimate the mean response, respectively. Heterogeneous variability along the ascending limb of the *H*-reflex recruitment curve would require that the mathematical analysis technique follows a sigmoid function. This hypothesis could also account for the observation that the sensitivity of the reflex response to facilitation and inhibition is a function of the size of the test reflex and would limit the use of a linear function to approximate the ascending limb (Crone et al. 1990).

Limitations of analysis techniques

The major limitations of fitting techniques are based on the required level of investigator manipulation, the response of the fitting techniques to the variability of the sampled data and the occurrence of repeated data points. Thus, certain analysis techniques evaluated in part I of the experiment were not used in the sensitivity comparison (part II).

All analysis techniques require a level of investigator control to properly fit the data. In the case of the linear, sigmoid, power and logarithmic techniques the peak of the ascending limb must be chosen to limit the data set for analysis. This study showed that there are no differences within curve fits with respect to the method of selection of the ascending limb of the recruitment curve. Conversely, the smoothing spline and polynomial analysis techniques do not require the selection of the peak of the ascending limb. These analysis techniques require fitting parameters to be subjectively set with the assistance of penalty features. The error fitting technique as well as limiting the curvature of the first or second derivative of the fitted curve are common penalty features used to justify alterations in fitting parameters (Hayes et al. 1979). However, the use of a penalty feature may have direct consequences on parameters of interest. For example, Christie et al. (2004) presented the first derivative of the 9th order polynomial fit to the HCRC as a measure of *H*-reflex excitability. The first derivative is a measure of the slope of the function at any stimulus intensity and is therefore a dynamic equivalent of the H_{SLP} presented by Funase et al. (1994a). However, altering the first or second derivative of the function is a direct manipulation of H_{SLP}, thereby introducing investigator bias with respect to this parameter of interest.

The polynomial and smoothing spline fitting techniques are highly sensitive to changes in the data, creating unnecessary curvature on the ascending limb which can cause erratic values for the parameters of interest. Also, the polynomial technique is unrestricted outside the bounds of the sampled data which can lead to erroneous results for parameters of interest such as H_{TH}.

The smoothing spline, logarithmic and power curve fits are not able to analyse recruitment curves with repeating data points. This severely limited the ability of these analysis techniques to analyse any HMRC data, and therefore these techniques were excluded from further analysis.

Ultimately the choice to evaluate the sigmoid curve fit alongside the accepted linear regression method in part II of the experiment was based on the fact that the HMRC analysis techniques were limited to the linear, sigmoid and polynomial techniques. Furthermore, the polynomial technique was also excluded due to difficulty in administration of the investigator controlled fitting parameter. A benefit of both the linear regression and sigmoid fits is that once the bounds of the data have been chosen these techniques are unaffected by experimenter bias (Carroll et al. 2001; Devanne et al. 1997; Funase et al. 1994a). Also, as previously discussed, both the linear and sigmoid curve fits are resilient to slight differences in the selection of the ascending limb of the RC for analysis. Therefore, a proper comparison of both analysis technique and presentation methodology with respect to parameters of interest was limited to the linear and sigmoid techniques.

Although we demonstrated that the response of a three parameter sigmoid function is better than other selected fitting techniques in the current study, other functions may be valuable tools used to ascertain changes in the *H*-reflex recruitment curve in different subject populations or experimental conditions. For example, it is necessary to consider that under pathological conditions there may be alterations in the physiological response of the *H*-reflex recruitment curve due to underlying morphological and functional changes. These changes may limit the ability of certain functions to fit the response properly. Subsequently, a five parameter sigmoid function may allow the determination of changes in the *H*-reflex that differentially affect the response characteristics at both the foot and the peak of the curve, respectively (Pitcher et al. 2003). It is important to note that observations in this study relate to the soleus reflex in intact subjects and may not encompass all subject populations or muscles studied. However, an identical sigmoid analysis was successfully performed on Flexor Carpi Radialis *H*-reflexes (Zehr et al. 2007b) and in stroke survivors (Barzi and Zehr 2007). Therefore, we are confident that the proposed method should prove robust across a variety of experimental settings.

Changes in parameters of interest with respect to analysis technique

The major result from the comparison of analysis techniques and data presentations from the movement conditioning experiments is that the HCRC sigmoid is the most sensitive to experimental differences in all parameters of interest and predicted values. Also, the HMRC sigmoid fit performed better than the HCRC linear and the HMRC linear analysis techniques. This suggests that even without measurement of current the sigmoid technique allows HMRC to be reliably analysed. The ability of the sigmoid technique to be more sensitive than the linear analysis technique to changes in parameters of interest and estimated values is a result of both the ability to set the bounds of the sigmoid technique and the accommodation of the sigmoid fitting technique to the different portions of the RC.

We predicted that only a limited portion of the RC follows a linear response. Crone et al. (1990) have shown that the susceptibility of a test reflex to facilitation or inhibition depends on the test reflex size and therefore its position on the ascending limb. This suggests that a linear function fitting the entire ascending limb may misrepresent different portions of the curve where responses may not be linear. Also, when using a linear function the data points chosen for linear regression normally exclude data points near the peak of the curve because they are thought to be affected by orthodromic collision (Funase et al. 1994a, 1996). Therefore, the upper limits of the data analysed using the linear fit technique is chosen just below the motor threshold ($M_{\mathrm{TH})}$ (Funase et al. 1994a, 1996). This results in a tendency to underestimate estimated values such as the $H_{@\mathrm{TH}}$ and overestimate values such as $H_{@\mathrm{MAX}}$. Evidence for this is apparent from both the movement conditioning and somatosensory conditioning where current at threshold, current at H_{MAX}, $H_{@\mathrm{TH}}$ and $H_{@\mathrm{MAX}}$ have rather variable and spurious values. This may have led to the exclusion of the use of these parameters in previous research (Funase et al. 1994a, b, 1996; Zehr and Stein 1999) For example, $H_{@\mathrm{TH}}$ for both movement and somatosensory conditioning for both HCRC and HMRC obtained through linear regression have negative values meaning that the intersection of the predicted value with the linear fit is negative. An apparent benefit of using the sigmoid curve fit is that this technique is bound in amplitude range by zero current or *M*-value and the maximum reflex response which is a predetermined input value (Carroll et al. 2001; Devanne et al. 1997). Restricting the sigmoid fit to both the zero and maximum reflex response at extreme ranges would serve to correct the potential overestimation at the foot of the curve and underestimation of the peak of the curve that is apparent in other techniques (Funase et al. 1994a).

It is evident that parameters of interest and predicted values show consistent results when analysed using the sigmoid technique. For example, for both movement and somatosensory conditioning experiments the current values at different *H*-amplitudes follow similar trends for both HCRC and HMRC. Comparing these results to the linear fit technique there are obvious disparities, such as when investigating the H_{MAX} value and the corresponding

$H_{@\mathrm{MAX}}$ value. The statistically significant result found for the $H_{@\mathrm{MAX}}$ during the somatosensory conditioning may be a result of exaggerated linear extrapolation. This trend is apparent when investigating the single subject data, as the slope of the conditioned linear fit RC exaggerates the maximum response.

Changes in parameters of interest with respect to presentation methodology

The results of this study suggest that the sigmoid is the most robust, simplest to administer and interpret, and follows physiologically based predictions of the stimulus response of the *H*-reflex recruitment curve. This is consistent when investigating HCRC; however, the analysis of HMRC requires a more thorough investigation of the parameters of interest. It is important to recognize that the *M*-wave is limited as a measure of stimulus intensity at both the foot and plateau of the MCRC. It is near these extreme ranges that a change in current intensity will fail to considerably affect the amplitude of the *M*-wave. Therefore, unless the HCRC falls within the middle range of the MCRC the ascending limb will have a large range of *H*-reflex amplitudes within a small range of *M*-wave amplitudes. This does not preclude its use in the determination of parameters of interest as shown from the consistent results from both the movement conditioning and the somatosensory conditioning experiments comparing HCRC to HMRC results. However, in some cases this may alter the interpretation of parameters of interest. For example, when examining the somatosensory conditioning experiment it is apparent that the HCRC results showed a significant change in current @50%H_{MAX} signifying a leftward shift in the RC. This same result is not seen in the HMRC results. Concurrently, there is a significant change in the slope in the HMRC that is not noticed in the HCRC. We suggest that this significant result seen as a change in the H_{SLP} in the HMRC is in fact a result of the HCRC shifting leftward with respect to the MCRC. This can be apparent when viewing the single subject RC for the somatosensory conditioning experiment.

Regardless of the technique used to analyse either the HCRC or the HMRC curve it is important to determine how changes in the input/output relation of each represent physiological changes when attempting to interpret results.

Conclusion

Mathematical analysis techniques have been used to create an approximate interpolation of the average responses on *H*-reflex RC so that certain parameters of interest can be obtained from the data. The sigmoid analysis technique may be a new reliable method used to investigate and compare both HCRC and HMRC. Greater approximation of parameters of interest with the use of the sigmoid analysis technique may provide more robust ways of interpreting experimental results.

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Appendix: Details of mathematical analysis techniques

Linear regression

For the linear fit, the ascending limb of the HRC was defined as all points from the foot of the curve to the current value that occurred at M_{TH} (approximately 10% below the peak of the HRC) by the manual placement of a cursor on the computer display (Funase et al. 1994a). For the cases where the M_{TH} occurred past the peak of the HRC the cursor was placed at approximately 10% below the peak of HRC. This was done to ensure that the slope of the line was not contaminated by *H*-waves near the peak of the ascending limb that could be potentially affected by collision along the motor axons (Funase et al. 1994a). For the linear curve fit the data from both the calc and the chos methods were fit using the least squares method which assumes that the variability in the reflex amplitude is Gaussian distributed. Equation 2 represents the linear fit model.

$$y = mx + b \tag{2}$$

where *x* is the stimulus intensity, *m* is the slope, and *b* is the intercept. The least squares linear regression finds *m* and *b* that best fit the sampled data by minimizing the value obtained using the least squares procedure. For detail of the least squares procedure and other possible linear optimization criteria see the National Instruments website (link above).

Polynomial

The polynomial fit technique finds the polynomial equation of the line that minimizes the mean square error from the fitted curve to the sampled data. Equation 3 gives the general form of the polynomial fit.

$$fi = \sum_{j=0}^{m} a_j x_i^j \tag{3}$$

where f represents the output sequence, x represents the input sequence, a represents the polynomial coefficients, and m is the polynomial order. The polynomial order is an arbitrarily chosen parameter that is under investigator control. Systematic increases in the polynomial order can cause random changes in the goodness of fit statistics. Also, the high order polynomials may obtain the best values of the goodness of fit statistics and yet be very noisy. Therefore, it is important to determine a method that defines the ideal polynomial order that produces an optimum combination of lowest mean square error while still producing a smooth curve. The technique used in this experiment is called "error fitting" and will be described below with reference to setting boundary conditions for both the polynomial and smoothing spline curve fits.

Smoothing spline

A cubic spline is a curve constructed of piecewise third-order polynomials which pass through a defined set of points. Analogous to a drawing device, a spline can be thought of as a flexible strip of material that may be bent into a curve and used to draw smooth curves between points. The HRC data was fit with a piecewise cubic spline (smoothing spline) function. This fits the sampled data by minimizing the following function:

$$p\sum_{i=0}^{n-1} w_i(y_i - f(x_i))^2 + (1-p)\int_{x_0}^{x_{n-1}} \lambda(x)(f''(x))^2 \mathrm{d}x \tag{4}$$

where p is the balance parameter, w_i is the ith element of weight. y_i is the ith element of the set of all normalized H-wave amplitudes. x_i is the ith element of the set of all normalized current amplitudes. $\mathrm{f}''(\mathrm{x})$ is the second order derivative of the cubic spline function, $f(x)$. $\lambda(x)$ is the piecewise constant function. The balance parameter (p) specifies the balance between the smoothness of the curve fit and the accuracy with which it fits the observations. If $p = 0$, the fitted model is equivalent to a linear model. If $p = 1$, the fitting is equivalent to cubic spline interpolation where all the data points are connected. The smoothing spline function parameters of data point weight and balance can be subjectively set by the investigator. A weight parameter can be set for each data point to define the relative importance of each data point towards the resultant curve fit. For the purpose of this experiment the weight of each point was considered equivalent, as each data point must have equal probability of defining the recruitment curve. Similar to determining the polynomial order, the balance parameter can be set by the investigator and therefore requires a method to determine the optimal combination of both smoothness and measures of goodness of fit. Increasing the balance parameter would both increase the correlation coefficient and decrease the root mean square error signifying an appropriate fit to the data. However, the fitted curve with a p of 1 would pass though every data point and therefore be very noisy. The balance parameter was adjusted to conform to two predetermined parameters to obtain the optimal combination of both smoothness and measure of MSE. The first penalty feature was the evaluation of the first and second derivatives of the developed fit. This measure would allow a determination of any rapid changes in the slope of the curve that would signify that the curve is too erratic. The second penalty feature was a variation of an idea first described by Hayes et al. (1979) described as "error fitting" in this manuscript.

Error fitting

During error fitting the adjustable parameter in either the polynomial or the smoothing spline technique is increased until the furthest point away from the fitted curve to any individual data point is no greater than the deviation value determined from the recruitment curve. The deviation value is determined using Eq. 5.

$$\sqrt{\frac{1}{N-1}\sum_{i=1}^{n-1}(y_{i+1} - y_i)^2} \tag{5}$$

Error fitting is thus sensitive to the greatest variability in the developed RC.

Power

The following equation represents the power fit model:

$$f = ax^b \tag{6}$$

where x is the input sequence, a is the amplitude, and b is the power. This curve fit finds a and b that minimizes the least square fit to the experimental observations.

Logarithmic

The following equation represents the logarithmic fit model:

$$f = a\log_c(bx) \tag{7}$$

where x is the input sequence, c is the base, a is the amplitude, and b is the scale. This fit finds a and b that minimizes the least squares fit to the experimental observations.

Sigmoid

A general least squares model of a custom three-parameter sigmoid function similar to one developed in TMS Research was used to fit the ascending limb of all recruitment curves (Carroll et al. 2001; Devanne et al. 1997).

$$H(s) = \frac{H_{\mathrm{MAX}}}{1 + e^{\mathrm{m(s50-s)}}} \tag{8}$$

where H_{MAX} is the upper limit of the curve, m is the slope parameter of the function, s50 is the stimulus at 50% of the H_{MAX} value, and H(s) is the *H*-reflex amplitude at a given stimulus value (s). Average H_{MAX} was calculated from the 5 largest peak-to-peak *H*-reflexes. The average H_{MAX} value (defined above) was used to define the upper limits of the sigmoid curve. The ascending limb of the recruitment curve was chosen as all points from zero current to a manually chosen peak of the recruitment curve.

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