A new approach for detecting and analyzing cutaneous reflexes during locomotion

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Bagna M, Bouyer LJ. A new approach for detecting and analyzing cutaneous reflexes during locomotion. J Neurophysiol 105: 1406–1415, 2011. First published December 1, 2010; doi:10.1152/jn.01095.2009.—During human walking, due to their small amplitude, individual cutaneous reflex responses are difficult to detect in surface EMG recordings. In this study, we present a new algorithm to automatically detect individual cutaneous reflex responses and to extract their corresponding onset latency, amplitude, duration, and sign. To discriminate reflex responses from the intrinsic variability of the background EMG, each stimulated cycle is compared with 10 adjacent nonstimulated cycles, looking for consistent differences. In the first 200 ms after stimulation, reflex responses are detected when ≥9/10 of these differences are either positive or negative. This approach does not require amplitude thresholds or fixed time windows for reflex detection. To reduce false detections, a postprocessing step selects 50 nonstimulated cycles randomly, processes them through the algorithm as stimulated cycles, and establishes a minimal reflex duration criterion that it then used to validate the detected responses. Validated responses from an entire test session are then reported on a colormap (reflex activity map) from which specific responses can be identified and quantified. The new method was validated in ten participants, three cutaneous nerves, and two protocols (phase modulation and recruitment curves). Compared with the classical method, the new algorithm showed better performance in terms of detection accuracy, specificity, and reliability. Although tested here to evaluate cutaneous reflexes during human walking, the simplicity of this method is such that it could easily be used with other reflexes, signals, and preparations.

STUDYING REFLEXES is one approach useful to improve our understanding of the organization of sensorimotor processing in humans. Cutaneous reflexes represent the link between skin afferents and motoneurons. During locomotion, cutaneous reflex responses are strongly modulated, going from excitation to inhibition from one phase of the gait cycle to another, a phenomenon called phase reversal (Duysens et al. 1990, 1992). In humans, cutaneous reflexes are signals of small amplitude, compared with those obtained in cats (e.g., Bernard et al. 2007) or with H-reflexes (e.g., Tucker et al. 2005). Identifying and quantifying them during walking has therefore been particularly challenging, because of the phase modulation of the responses and of the large variations in background EMG activity present within a gait cycle.

The classical solution to improve signal-to-noise ratio has been to divide the gait cycle into 16 bins of equal width and to average several reflex responses obtained in each of these bins [stimulus-triggered averaging (STA); Yang and Stein, 1990; Duysens et al. 1990, 1992; Van Wezel et al. 1997]. Typically, 10–30 responses are averaged in each bin. As already pointed out by others (Brinkworth and Turker, 2003), STA presents some limitations: because individual EMG responses to the same stimulation are all somewhat different (Lavigne et al. 1983), averaging tends to blur the responses in terms of exact latency, amplitude, and duration. In addition to STA, the classical method uses fixed time windows to quantify cutaneous reflex responses (Yang and Stein, 1990; Duysens et al. 1990, 1992; Van Wezel et al. 1997). During walking, as reflex responses at given gait phases change from monophasic to biphasic (e.g., see Yang and Stein 1990, Fig. 4), averaging such responses over a fixed time window can drastically lower the reported response strength. Taken together, these factors show the need to develop computer algorithms to objectively and accurately quantify individual cutaneous reflex responses during walking.

In the present study, we introduce an original method to analyze cutaneous reflexes during walking that does not use STA or fixed time windows. To discriminate reflex responses from the intrinsic variability of background EMG, each stimulated cycle is compared with several adjacent nonstimulated cycles, looking for consistent differences. With this method, individual responses evoked by stimulation can be quantified. There are three aims to the present study: 1) to present a reliable and repeatable method for cutaneous reflex response processing that can accurately estimate individual response latency, duration, and strength; 2) to show that the proposed method performs better than the classical approach (Yang and Stein 1990; Duysens et al. 1990, 1992; Van Wezel et al. 1997) in terms of detection accuracy, specificity, and reliability; and 3) to demonstrate that the algorithm can also be used for other purposes, in this case objectively measuring the motor threshold for cutaneous reflex stimulation, another situation in which classical methods tend to be limited. Preliminary results have appeared in abstract form (Bagna and Bouyer, 2008).

METHODS

Subjects

A total of 23 experiments were performed in 10 healthy subjects (age range 23–35 yr) with no reported neurological or major orthopedic history. All participants gave informed consent to the protocol, which had been previously approved by the local ethics committee.

Experimental Procedure

Subjects came to the laboratory for one to three visits (one visit per nerve). Three cutaneous nerves with receptive fields (RF)
located on the foot were stimulated: superficial peroneal (SP, RF = foot dorsum), sural (SU, RF = lateral aspect of the foot), and tibial (TIB, RF = foot sole). On each visit, subjects walked on a motorized treadmill at constant speed (4 km/h) for a period of 9 min. Pairs of disposable electrodes (Kendall SOFT-EH69P; 2.5 cm apart) were used for transcutaneous stimulation of the nerves. For SP stimulation, the electrodes were placed distal to the superior extensor retinaculum and posterior to the tendon of the extensor digitorum longus muscle. For SU stimulation, the electrodes were placed posterior to the lateral malleolus. Finally, for TIB stimulation, they were located over the flexor hallucis longus muscle, posterior to the medial malleolus. Electrical stimulation consisted of a train of six pulses (pulse width = 1 ms) delivered at 200 Hz once every two to three strides and were produced by a Grass S88 stimulator through an isolation box (Grass SIU5) and a constant current unit (Grass CCU1).

EMG activity was recorded from the tibialis anterior muscle (TA, ankle dorsiflexor) via surface EMG electrodes (Kendall Meditrace 200) placed in a bipolar configuration. 2 cm apart, according to the surface electromyography for the noninvasive assessment of muscles (SENIAM) project recommendations (Hermens et al. 2000). EMG activity was band-pass filtered at 10–500 Hz and sampled at 1,000 samples/s before acquisition. Heel strike (HS) data were collected by use of custom-made footswitches. Stimulus intensity was recorded by using a custom-made stimulus integration circuit.

Two experimental protocols were tested. 1) Modulation protocol: 160 stimulations were delivered at different moments with respect to HS to cover the entire gait cycle. The intensity of stimulation was set between 2.5 and 3 times the radiating threshold (level at which the subject begins to feel radiation from the stimulation electrodes toward the foot) and was kept constant over the whole test session. To reduce predictability, stimulation occurred every second or third stride, and the moment of stimulation within the gait cycle followed a pseudo-random sequence. 2) Recruitment protocol: this time, the phase of stimulation was kept constant (90% of the gait cycle, i.e., late swing) while stimulation intensity was varied from below the radiating threshold to mild discomfort (range 2–12 mA; maximum of 4 mA).

A total of 160 stimulations were delivered, once every two to three strides to reduce predictability.

New Cutaneous Reflex Processing Algorithm

Figure 1 presents a block diagram of the new algorithm developed for cutaneous reflex processing. It can be separated into four elements: preprocessing, individual reflex detection, individual reflex identification, and postprocessing. Each element is described separately below.

Preprocessing. For each 9-min walking session, the TA EMG signal is rectified and low-pass filtered by use of a nine-point moving average filter, to extract the EMG activity envelope. The signal is then divided into gait cycles on the basis of HS information. Each gait cycle is tagged as stimulated (STIM) or nonstimulated (NO-STIM) on the basis of the stimulus intensity channel. When stimulation occurs within the last 200 ms of a STIM cycle, the subsequent gait cycle is also considered as a STIM cycle because of the potential presence of a long latency response starting around 170 ms (see Yang and Stein 1990).

Individual reflex detection. To detect the presence of a reflex, each STIM cycle is compared with N NO-STIM cycles looking for consistent differences. In the present study, the 10 NO-STIM cycles preceding each STIM cycle were used (N = 10). The justification for this number can be found in the appendix.

The reflex detection process is presented in Fig. 2 and can be summarized by the following four steps:

1) For each STIM cycle, the preceding 10 NO-STIM cycles are selected and time normalized to STIM cycle duration (Fig. 2A).

2) Each one of the time-normalized NO-STIM cycles is subtracted sample-by-sample from the STIM cycle, creating 10 difference signals Δ_Si where 1 ≤ i ≤ N (Fig. 2B).

3) Analysis is performed in the interval ranging from 40 (to avoid contamination from the stimulus artifact) to 200 ms after stimulation. Let N equal the number of subtracted gait cycles, and Δ_Si (1 ≤ i ≤ N) represent the difference signals. For each sample k from 40 to 200 ms, the confidence index value (CIV) is calculated as

\[ CIV(k) = \frac{\sum_{i=1}^{N} [\Delta_S(k) > 0]}{N} \quad (1) \]

where 0 ≤ CIV ≤ 1 (Fig. 2C).

For each sample k, the CIV measures the agreement among the difference signals (Δ_S) on the presence and sign (excitation/inhibition) of a potential reflex response, simply by counting the proportion of difference signals that are positive and negative. Indeed, for each difference signal Δ_S, the samples are equal to NO-STIM EMG activity have a value of 0, those above NO-STIM EMG activity have a positive value, and those below NO-STIM EMG activity are negative. This implies that if CIV(k) = 1, then 100% of the difference signals agree that the STIM signal at sample k is above 0, i.e., above NO-STIM EMG activity. This sample is therefore considered to represent an excitation. Conversely, when CIV(k) = 0, all difference signals agree that sample k is below the NO-STIM EMG level, i.e., that it represents an inhibition. Finally, when CIV(k) = 0.5, then the difference signals are equally distributed around 0. There is no agreement between them on the sign of a potential response, and the sample is therefore considered to be no different from the NO-STIM EMG activity. In this study, because 10 difference signals are used by the algorithm, the CIV value varies between 0 and 1 in steps of 0.1.

4) To reliably identify reflex responses, we chose that 9 of 10 difference signals had to agree on the sign of the response (level of confidence set at 90%; P = 0.9). In practical terms, this means that when CIV(k) ≥ 0.9 an excitation is detected, and when CIV(k) ≤ 0.1 an inhibition is detected (Fig. 2C).}

Individual reflex identification. Seven parameters were defined to characterize each detected reflex response.

PARAMETER 1: PHASE OF STIMULATION. The phase of the gait cycle in which the stimulation occurred. The phase of stimulation was calculated in percentage of the gait cycle:

\[ \varphi(\%) = 100 \times \frac{\text{time of stimulation onset}}{| \text{reflex cycle duration} |} \quad (2) \]

PARAMETER 2: SIGN. Responses are classified as excitations or inhibitions on the basis of their CIV (see reflex detection above). A CIV(k) ≥ 0.9 represents an excitation. A CIV(k) ≤ 0.1 represents an inhibition.

PARAMETER 3: ONSET LATENCY. Onset latency is defined as the point in time when the CIV reaches 0.9 (selected P value; see reflex detection above) for an excitation or 0.1 (1 − P) for an inhibition.

PARAMETER 4: RESPONSE DURATION. Response duration is defined as the number of consecutive samples in which the CIV remains ≥0.9 for an excitation, or ≤0.1 for an inhibition.

PARAMETER 5: RESPONSE AREA. Response area is defined as the area under the mean of the difference signals (calculated by the trapezoid method) over the duration of the detected reflex. Area is positive when the reflex is an excitation and negative when it is an inhibition (Fig. 2D).

PARAMETER 6: RESPONSE AMPLITUDE. Response amplitude is defined as the response area divided by its duration. Amplitude is positive when the reflex is an excitation and negative when it is an inhibition (Fig. 2D).

PARAMETER 7: BACKGROUND EMG ACTIVITY. Background EMG activity represents an estimation of the motoneuronal pool excitability at the time when the reflex response occurred. The 10 NO-STIM cycles associated with a given STIM cycle are first averaged together.
New algorithm for cutaneous reflex processing. It is divided into 4 parts: EMG preprocessing, reflex detection, reflex identification, and postprocessing. The raw EMG signal is rectified and filtered before gait cycles are divided from one heel strike (HS) to the next. Stimulated (STIM) and nonstimulated (NO-STIM) cycles are then identified and tagged. Every STIM cycle is compared with the preceding 10 NO-STIM cycles. Ten “difference signals” are constructed by subtracting each of the 10 NO-STIM from the STIM cycle. A confidence index value (CIV) is calculated every millisecond from 40 to 200 ms poststimulation to estimate the probability of the presence of a reflex response (see text for more details). By use of this CIV, individual reflex responses are identified and characterized with their respective onset latency, duration, and strength (amplitude and area) and stored in a 2-dimensional matrix. Finally, postprocessing consists of removing all responses shorter than a given duration criteria (based on a false positive responses estimation procedure; see text and Fig. 3). Finally, background EMG activity at the time when the reflex response occurred is estimated from the average of the 10 NO-STIM cycles associated with a given STIM: the mean amplitude of this average is taken from response onset to response end.
Background EMG activity is then defined as the mean amplitude of this average taken from response onset to response end.

Postprocessing. Postprocessing was performed to increase further detection reliability by estimating and reducing type I errors (false detections). First, to estimate false detections, 50 NO-STIM cycles are randomly selected from the 9-min test session. Each one of these cycles is then processed through the algorithm as if it were a STIM cycle. In this situation, any response detected by the algorithm represents a false positive, because it did not result from a real stimulation. A frequency histogram (no. of occurrences vs. response duration; Fig. 3) is then constructed from these false positive responses to establish a minimal reliable response duration. In the representative subject illustrated in Fig. 3 (subject 2), the minimal reliable response duration was set at 13 ms, since 85% of the false positive responses fell below this value. Secondly, all reflex responses detected in the real STIM cycles are scanned by the algorithm. Responses with a duration below the minimal reliable duration (13 ms in this example) are discarded. The remaining reflex responses are referred to as validated responses.

Classical Cutaneous Reflex Processing Algorithm

For comparison purposes, the data collected as part of this study were also processed by using a previously published method used for cutaneous reflex analysis (Yang and Stein 1990).

It can be summarized by the following seven steps:

1) The recorded EMG signals are divided into individual gait cycles by using HS information and are classified as STIM or NO-STIM on the basis of the presence or absence of stimulation, respectively.

2) All NO-STIM cycles are rectified, time normalized to mean NO-STIM cycle duration, and averaged together to represent the mean background EMG activity present over the whole test session.

3) The gait cycle is divided into 16 bins of equal width. STIM cycles are assigned to one of these bins according to the time of arrival of stimulation in the gait cycle.

4) For each bin, STIM cycles are rectified and synchronized at stimulus onset, and the mean background EMG activity (point no. 2 above) is removed. Responses are then averaged together to produce a mean reflex response.

Fig. 2. Example of cutaneous reflex analysis for one stimulated cycle evoked in the tibialis anterior (TA) muscle during swing (sural nerve stimulation, subject 2). A: rectified and filtered stimulated cycle (black line), superimposed on the 10 preceding nonstimulated cycles (gray lines). The stimulus artifact and 85-ms response are shown by the arrows. B: 10 difference signals constructed by subtracting each of the 10 NO-STIM from the STIM cycle. C: expanded view of the first 200 ms after stimulation, superimposing the CIV (dark line) on top of the difference signals (gray lines). The first 40 ms after stimulation are not considered for CIV calculation to avoid contamination from the stimulus artifact. Note that the y scales for the CIV and difference signals are different. Arrows show that the CIV value discriminates between excitations (CIV > 0.9) and inhibitions (CIV ≤ 0.1). D: validated response obtained after postprocessing. It includes several excitatory and inhibitory components, each characterized by its own onset latency, duration, and strength (amplitude and area).
5) This process produces 16 average reflex responses, one for each bin, summarizing the reflex activation pattern present over the gait cycle.

6) The first 200 ms after stimulation are analyzed.

7) A fixed time window for reflex measurement is set by visual inspection from the experimenter, trying to accommodate responses in all bins while minimizing response truncation. Mean reflex amplitude is calculated over the entire time window length (1 output value per bin).

The two algorithms were implemented by use of the MatLab software (MathWorks, Natick, MA).

Data Analysis

As individual responses from 160 stimulations are recorded and parameterized for each 9-min walking session in this study, a practical means of graphically presenting such a large amount of data had to be developed. Plots named reflex activity maps (RAMs) were therefore created.

To create a RAM of an entire recording session, validated responses from each STIM cycle are plotted for the first 200 ms after stimulation on a single horizontal line. Reflex amplitude is represented by a color scale (Fig. 4B); all 160 responses are stacked, ordered as a function of phase of stimulation in the gait cycle, with the bottom trace representing a phase of 0% (i.e., HS).

Since RAMs can easily represent such a large amount of information, they were also used to present unprocessed reflexes for comparison. An example is shown in Fig. 4A. In the unprocessed RAM, each line now represents the average of the 10 difference signals (Δ), instead of the responses detected and validated by the new algorithm.

RESULTS

Reflex Modulation Experiments

Figure 4 compares the output of the new (Fig. 4B) and classical (Fig. 4C) algorithms and relates them to the unprocessed reflexes (Fig. 4A). By comparing the validated responses with the unprocessed reflexes, it can be seen that the detection process does not lose information or distort the raw data but enhances the signal-to-noise ratio of the individual responses. Compared with the 16 average reflex responses obtained with the classical method, it is clear that the new method preserves much more details from the raw data. For example, the gradual phase advance in the onset of the inhibition found around 110 ms at 80% of gait is visible in the validated RAM, but not in the average reflex responses of Fig. 4C. It can also be noted that response amplitude tends to be larger with the new method (more...
details regarding amplitude comparisons will be provided in subsequent figures).

Looking along the gait phase axis (y-axis) of the validated RAM, it can be seen that stimulations given in the first 50% of the gait cycle produced only a few low-amplitude detections whereas stimulations delivered in the last 50% produced a large number of detections. This is consistent with what is reported at lower resolution with the classical method, where reflex responses become visible only at bin 9 and phase reversal is seen around 85 ms between bins 14 and 16. It must be noted that although validated responses were based on detection criteria \((CIV \geq 0.9)\) and removal of short-duration responses associated with false positives; see METHODS), the classical method is simply a mean trace without any information about the actual spread of the individual reflex responses that were actually averaged.

**Detection of specific responses.** The two methods also differ in the way reflex responses are extracted and quantified. With the new method, vertical cursors are manually placed on the validated RAM. All responses that are in contact with this cursor are tagged, regardless of their exact onset latency. This way, responses occurring around a given latency can be identified while preserving their individual onset latency, duration, and amplitude.

As an example, a cursor set at 85 ms on Fig. 4B tags responses in a range that would be called “middle latency” (Yang and Stein 1990). The following parameters of each tagged response are reported in Fig. 5, left (solid circles): background EMG activity, mean amplitude, onset latency, and response duration. Only a few low-amplitude responses are detected when stimulation occurs in the first 50% of the gait cycle. Then, a large and consistent excitatory response is observed between 50 and 80%, followed by a clear inhibition (reflex reversal) in the last 20%. These results concur with a visual examination of the validated RAM (Fig. 4B). The reported individual onset latencies spread from 52 to 84 ms, depending on the phase of gait. Response duration ranged from 14 to 49 ms.

By using the classical method to measure an equivalent response, a time window going from 70 to 110 ms was visually

![Fig. 5. Quantitative comparison between the new and the classical methods of cutaneous reflex analysis for a typical subject (2). Estimated background EMG activity (1st row), response amplitudes (2nd row), onset latencies (3rd row), and response durations (4th row) presented as a function of phase of the gait cycle for sural (SU) nerve stimulation at 2.5 × radiating threshold (RT) during walking in subject 2. Responses were recorded in the TA muscle. Left: 85-ms response extracted by using the new algorithm \((\bullet)\) superimposed on a middle latency response extracted with a 70- to 110-ms fixed time window using the classical method \((\odot)\). Right: 125-ms response extracted by using the new algorithm \((\bullet)\) superimposed on a response extracted with a 111- to 160-ms fixed time window by the classical method \((\odot)\).](http://jn.physiology.org/doi/10.1152/jn.01179.2010)
chosen as the best compromise, trying to include responses in all bins while at the same time avoiding response truncation. The parameters of each response are reported in Fig. 5, left (open circles), superimposed on the individual responses obtained with the new method (solid circles) to allow comparison. By setting a fixed time window, all responses have the same onset latency (70 ms) and duration (40 ms).

Comparing the two methods, it can be seen that the amplitude modulation of reflex responses as a function of phase keeps the same profile. However, the new method yields responses that are of higher amplitude, delayed onset latency, and shorter duration. These findings are not surprising, since the constraints associated with the old method force the user to define window onset on the basis of the bin having the response with the shortest onset latency, and window end on the basis of the bin with the response ending last. As a result, averaging in individual bins occurs over more than the duration of actual responses, thereby underestimating mean amplitude (see Fig. 4C). In addition, as mentioned in the Introduction, the process of averaging responses with slightly different individual onset latencies will also tend to “flatten” the signal, further reducing the measure of mean reflex amplitude. In contrast, the new method is not affected by the flattening effect resulting from response averaging or by the constraints associated with setting a fixed time window. As a result, individual responses are quantified more accurately not only regarding actual onset times and response durations, but also in terms of amplitude measurements.

As another example, a cursor was manually placed at 125 ms (see Fig. 4B). Results are presented in Fig. 5, right. Solid circles represent individual responses obtained with the new method. Open circles represent responses obtained with the classical method using a time window going from 111 to 160 ms.

Contrary to the 85-ms response, the 125-ms response shows an inhibition at the beginning of swing, reversing to an excitation in the last 15% of the gait cycle. Onset latency of individual responses ranges from 77 to 119 ms. Response duration ranges from 14 to 74 ms. Here also, the responses obtained with the classical method have lower amplitudes and lack details regarding onset latency and duration.

Validation of the New Algorithm for Different Nerves and Different Subjects

The new algorithm was tested in several subjects and different nerves: SU nerve (6 subjects), SP nerve (6 subjects), and TIB nerve (10 subjects). Results for the SU nerve stimulation showed consistent 85-ms latency response modulation across subjects: all participants presented a phase-dependent reversal. The response was mainly excitatory from 50 to 80% of the gait cycle and then became inhibitory. Only a few small responses were detected in the first 50% of the gait cycle. These results are consistent with the ones reported by others (Yang and Stein 1990; Duyssens et al. 1992; van Wezel et al. 1997; Zehr et al. 1998). The 85-ms response modulation to SP nerve stimulation was not as consistent across subjects. Among the six subjects tested, only three showed a reversal; in one of them, the reflex response was first inhibitory, then excitatory. Finally, in the ten subjects in which the TIB nerve was stimulated, six presented only inhibitory responses, whereas the other four showed phase-dependent reversals. This finding is also consistent with previous studies (Yang and Stein 1990; van Wezel et al. 1997).

To quantify and to validate statistically the improvement in performance obtained when using the new algorithm, the size of the reflex responses measured by the two methods was compared. To be as objective as possible, the most reliable response found in all three nerves was chosen (onset latency ~85 ms). Data from all subjects presented in the previous paragraph were processed through both algorithms. A mean reflex amplitude per test session was obtained for each method by taking the absolute value of all responses, regardless of phase. Since the same data sets are used by the two algorithms, no bias is introduced and the two methods can be safely compared. Figure 6 presents the group results. The improvement in performance is very significant. For SU, mean reflex amplitude went from 15.6 ± 6.1 to 36.7 ± 4.0% of peak background EMG, more than doubling ($P < 0.01$, paired t-test). For SP and TIB, mean reflex amplitude went from 7.9 ± 2.0 to 29.2 ± 2.1% ($P < 0.01$) and 4.9 ± 0.5 to 31.4 ± 3.1% of peak background EMG ($P < 0.01$), respectively. Although the size of the improvement varied slightly across subjects, all presented an improvement with the new method.

Recruitment Experiments

To define the relationship between stimulus intensity and motor response, the timing of the stimulus in the gait cycle was kept constant, while its intensity was varied (see METHODS). A plot of response size as a function of stimulus intensity is called a recruitment curve. Recruitment curves have many functions (see DISCUSSION), including providing information on the motor threshold of a given neural pathway. Another feature of the new algorithm is to enhance our ability to make recruitment curves for small responses such as human cutaneous reflexes. As each evoked reflex can be quantified individually, it is easy to follow the evoked responses pattern produced by gradually increasing stimulus intensity. To do this, a RAM is constructed, but this time with responses ordered vertically as a function of stimulation intensity rather than phase of gait. The Fig. 7A RAM presents unprocessed reflex responses obtained in TA when stimulating the SU nerve at 90% of the gait cycle (late-swing) and when varying the stimulus intensity from 2 to 12 mA in subject 2. Figure 7B shows the corresponding validated responses. As already shown.

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for the phase modulation experiment, the validated responses are faithful to the raw data. The RAMs show two main evoked responses, an inhibition around 100 ms followed by an excitation around 140 ms. For the inhibition, as stimulus intensity is increased, it can be seen that response duration increases more than response amplitude. This finding, obtained directly from looking at the RAM, suggests that response area is a better indication of motor pool recruitment than mean amplitude in this example. Extracted responses obtained by placing cursors at 100 and 140 ms are presented in Fig. 7, C and D, respectively. It can be seen that although the area and duration of the inhibition covary, the excitation follows a different pattern, in which duration remains constant but area increases. These results suggest that care must be taken when studying biphasic responses, since their two components (the excitation and the inhibition) may be differentially modulated.

DISCUSSION

Performance of the New Algorithm: Comparison with a Classical Method

The new algorithm presented in this study was validated in 10 subjects and was compared with a classical method used for cutaneous reflex analysis during human walking (e.g., Yang and Stein 1990; Duyssens et al. 1990, 1992; Van Wezel et al. 1997). It outperformed the classical method in terms of detection accuracy, specificity, and reliability. As shown in Figs. 4 and 5, the ability to detect individual response latency and duration rather than using fixed time windows improves detection accuracy as shown by shorter responses having higher amplitudes (Figs. 5 and 6). The reasons for these higher reflex amplitudes are that 1) using fixed windows includes portions that do not have a reflex component or that have responses of opposite sign (see Fig. 4C) and 2) averaging responses having slightly different onset latencies to obtain a bin average flattens out the response. In addition, the fact that the new algorithm can analyze each response individually produces at least 10 times more reflex responses within a given test session than methods based on STA, since STA requires at least 10 reflexes to be averaged to improve signal-to-noise ratio (see Duyssens et al. 1990). Since all response parameters are now conserved, data analysis can be more specific. For example, the new method allows seeing the relationship between response onset latency and phase of stimulation (Fig. 4B), something that could not be studied before because of the large phase difference between the 16 bins that were used to describe the gait cycle. Finally, another important improvement with the new algorithm is the reliability of the reflex detection process. Since no subjective input is required during processing, and since the algorithm involves statistical identification (voting; see Original Features of the New Algorithm) rather than using thresholds, the consequence is that if a given data set is run through the algorithm several times, the validated responses will always be the same. The use of the CIV for detection also provides a systematic approach that uses objective criteria for all data sets processed, thereby eliminating interexperimenter variability, a factor that can greatly affect the size of the measured reflex responses (Lavigne et al. 1983). To the best of our knowledge, our new algorithm is currently the only...
method available to perform such a detailed analysis of cutaneous reflex responses during human walking.

Original Features of the New Algorithm

There are several new elements that were introduced as part of the new reflex processing algorithm presented in this study that participated to the improvement in performance described above, probably the most important one being the CIV.

The CIV allows individual reflex responses to be detected with a set level of confidence (here 90%) by using neighboring nonstimulated cycles (here 10) as a reference. When at least 9 of 10 of the individual comparisons between STIM and NO-STIM cycles (difference signals) are either positive or negative, a reflex is detected. This “voting” approach has the advantage of not requiring a threshold for reflex detection and of taking into account the varying level of background activity that is present at different phases of the gait cycle. By nature, this method will self-adjust to each muscle activation profile (e.g., flexor vs. extensor) and to each participant.

The CIV is also useful for reflex response identification, serving to retrieve the exact onset latency, duration, and sign of detected responses. Finally, the CIV is used as a tool to reduce false positive detections (type I errors; Fig. 3). The calculation of the CIV is so simple (see Eq. 1) that it can easily be used for other types of reflexes or signals and can likely help experimenters by adding statistical confidence in their measurements of small responses evoked during movement.

Another element developed as part of the new algorithm that has implications for future applications is the background EMG activity estimation based on a limited number of adjacent NO-STIM cycles. Simulations presented in the Appendix show that typical reflex responses can be identified by using as little as 10 NO-STIM cycles as a reference. This feature can become very useful in situations in which reflex evaluations have to be made within a short-duration recording session such as for reflex evaluations in pathological gait (low subject endurance) or when background EMG is rapidly changing such as in motor adaptation studies (e.g., Blanchette and Bouyer, 2009). Also, individual background EMG activity estimations such as presented in Fig. 5 (top row) allow for precise measurements of motoneuronal pool excitability near the time of reflex detection. This feature can simplify the evaluation of automatic gain control contribution to reflex response modification when comparing test sessions obtained at different levels of baseline EMG (e.g., Bagna et al. 2010).

Because of the large amount of data having to be presented (160 reflexes with multiple responses), this study introduced a new way of globally looking at human cutaneous reflex responses during a whole walking session: RAMs. RAMs present simultaneously the 160 reflex responses, with a 1-ms resolution over the 200-ms following stimulation. They are constructed without making any assumptions about reflex amplitude, onset latency, duration, sign, or number of responses to a single stimulation. RAMs keep number of responses, phase, and time information on the same figure, allowing multiple comparisons to be made easily between different phases within a given test session or across sessions or subjects. In modulation experiments, RAMs show amplitude modulations (including reversals) together with the phase at which they occur. In recruitment experiments, RAMs show the relationship between stimulus intensity and response characteristics. RAMs can also help identify when mean amplitude or area is the most appropriate variable to describe a response (e.g., Fig. 7).

Reexamination of Reflex Modulation Experiments

Classical studies of cutaneous reflex modulation during human walking have reported three specific responses evoked by nerve stimulation: an early (55 ms), a middle (80 ms), and a long (170 ms) latency response (Yang and Stein 1990). Looking at RAMs more carefully shows that trying to describe human cutaneous reflexes with only three responses is an oversimplification. The availability of this new graphical representation coupled to quantitative evaluation with the new algorithm allows reflex analysis to be pushed further. In our opinion, reflex modulation studies must therefore go beyond describing three particular responses and their modulations. They should also include a level of analysis in which the entire evoked activity is assessed globally. RAMs provide the means of performing such a meta-analysis. Furthermore, we hypothesize that RAMs could even become a useful tool in the assessment of pathological reflex processing, by comparing the patients’ RAMs to a reference RAM constructed from a bank of normal subjects.

Fig. A1. Sensitivity test. A simulated square reflex response was introduced on a NO-STIM cycle at 750 ms after HS (within the swing-phase TA activity; simulating a worse case scenario) and put through the reflex analysis algorithm while the number of NO-STIM cycles composing the background EMG activity was varied. Mean errors on the detected amplitude (A), onset latency (B), and duration (C). It can be seen that >10 NO-STIM cycles are sufficient for reliable detection.
Recruitment Curves

Recruitment curves (experiments in which the phase of stimulation was fixed and stimulus intensity varied) are very useful in defining motor threshold as well as other parameters related to the state of the motoneuron pool and the efficacy of transmission through the pathway under study (see Capaday 1997 for a review). During walking, recruitment curves (also called input-output curves) have been used mainly in studies involving motor evoked potentials (e.g., Capaday 1997). Because of their lower amplitude, cutaneous reflexes evoked in humans during walking have been more difficult to work with and have therefore received less attention in this protocol. This is unfortunate, since animal model experiments have shown a large change in the motor threshold and steepness of the recruitment curve after cutaneous denervation in cats (Bernard et al. 2007), suggesting that important information not directly available in phase modulation experiments can be obtained with recruitment curves. The ability of the new algorithm to extract individual reflex responses with confidence (P ≥ 0.9) now makes it possible to study recruitment curves for cutaneous reflexes in humans. With tools such as validated RAMs in which reflex responses are ordered as a function of stimulus intensity (Fig. 7), one can now consider setting stimulation threshold based on quantitative measurements of motor threshold rather than using perceptual means such as the radiating threshold. In addition, recruitment curves could be used to measure changes in cutaneous reflex pathways during walking associated with pathology or motor learning that cannot be addressed with modulation experiments (e.g., motor threshold, slope, and peak value) and relate them to what has been reported after lesions of the nervous system in animal models (Bernard et al. 2007). This application of the new algorithm will also widen our understanding of processing in human cutaneous reflex pathways during movement.

Other Potential Applications of the New Algorithm

Although the new method presented here has been validated in the context of human cutaneous reflex detection and identification during walking, it is our belief that it can also be adapted to other types of reflexes [e.g., reciprocal inhibition (Kido et al. 2004), stretch reflex (Sinkjaer et al. 1996), unload reflex (Grey et al. 2004)], other types of electrophysiological signals (e.g., electro-neurograms, electroencephalograms), and other preparations (e.g., animal models). The only requirement is that data be recorded for a cyclical task (e.g., walking, breathing, chewing). In addition, it should be noted that the algorithm was designed to be easily adaptable for online use; this is why only preceding NO-STIM cycles are used for response identification. This feature widens even more the use of our new algorithm, toward protocols that require real-time data analysis such as reflex conditioning (e.g., Wolpaw and Tennissen, 2001).

APPENDIX

Sensitivity Test

To calculate the minimal number of NO-STIM cycles that are necessary for a reliable reflex identification, a simulated reflex response (square wave; 50-ms duration; 90-µV amplitude; 750 ms after HS) was added to a randomly chosen background cycle of a normalized length of 1,200 ms. This simulated reflex was inserted at a time when TA is active during the swing phase (worst case scenario). The simulated reflex was passed through the detection algorithm with a number of associated NO-STIM cycles ranging from 2 to 50. For each situation, the detection process was run 10 times, each time using randomly chosen NO-STIM cycles. The mean error over the 10 runs was calculated for amplitude, onset latency, and duration. The results are shown in the figure below. As can be seen from Fig. A1, the mean error on amplitude, latency, and duration estimation stabilizes when the number of NO-STIM cycles reaches 10.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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