Human stretch reflex pathways reexamined

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Yavuz ŞU, Mrachacz-Kersting N, Sebik O, Ünver MB, Farina D, Türker KS. Human stretch reflex pathways reexamined. J Neurophysiol 111: 602–612, 2014. First published November 13, 2013; doi:10.1152/jn.00295.2013.—Reflex responses of tibialis anterior motor units to stretch stimuli were investigated in human subjects. Three types of stretch stimuli were applied (tap-like, ramp-and-hold, and half-sine stretch). Stimulus-induced responses in single motor units were analyzed using the classical technique, which involved building average surface electromyogram (SEMG) and peristimulus time histograms (PSTH) from the discharge times of motor units and peristimulus frequencygrams (PSF) from the instantaneous discharge rates of single motor units. With the use of SEMG and PSTH, the tap-like stretch stimulus induced five separate reflex responses, on average. With the same single motor unit data, the PSF technique indicated that the tap stimulus induced only three reflex responses. Similar to the finding using the tap-like stretch stimulus, ramp-and-hold stimuli induced several peaks and troughs in the SEMG and PSTH. The PSF analyses displayed genuine increases in discharge rates underlying the peaks but not underlying the troughs. Half-sine stretch stimuli induced a long-lasting excitation followed by a long-lasting silent period in SEMG and PSTH. The increase in the discharge rate, however, lasted for the entire duration of the stimulus and continued during the silent period. The results are discussed in the light of the fact that the discharge rate of a motoneuron has a strong positive linear association for the entire duration of the stimulus and continued during the silent period. The peaks in the EMG, there are also silent periods between the peaks that can only be observed if the studied muscle has some background activity preceding the stretch stimulus (Matthews 1984; Miles et al. 1995; Poliakov and Miles 1994).

To date, the methods used to study stretch reflexes are the probabilistic methods based on trigger-averaged surface electromyography (SEMG) and the peristimulus time histogram (PSTH). Fundamentally, trigger-averaged SEMG and PSTH present the correlation of gross muscle and single motor unit activity against the stimulus timing, respectively. Whereas the SEMG is a conventional method that is also used for clinical diagnosis, the lesser known PSTH method is a histogram that shows the number of motor unit spike occurrences in a particular time window around the stimulus (Davey et al. 1986). However, recently, there have been a number of studies suggesting that the probabilistic methods distort the data significantly and mislead the investigator regarding the sign, amplitude, and shape of the stimulus-evoked net synaptic potential underlying the reflex response (Awiszus et al. 1991; Türker and Cheng 1994; Türker et al. 1996). To test these assertions directly, known currents have been injected into regularly discharging motoneurons in rat brain slices and the spike output analyzed using the probabilistic methods (Türker and Powers 1999, 2003). These experiments illustrated that the probability-based methods, such as PSTH and SEMG, can be misleading in representing the input (synaptic) current. The underlying reason is that these methods are influenced not only by the input current but also by the autocorrelation factor of the underlying spikes (Türker and Powers 1999, 2003, 2005). The action potentials, which are advanced or delayed with excitatory or inhibitory postsynaptic potentials (PSPs), occur again synchronously after one interspike interval. Since these probabilistic methods rely on the probability of spike occurrence, these consecutive responses can be erroneously interpreted as long- or medium-latency reflex responses.

SIR CHARLES SHERRINGTON in 1924 observed that a lengthening of a muscle will cause that muscle to contract (Liddell and Sherrington 1924). This behavior is also referred to as the stretch reflex and has since been utilized as both a diagnostic and research tool (Marsden et al. 1976; Matthews 1986). In the former, the integrity of the nervous system along the various levels of the spinal cord may be tested when a spinal cord lesion is suspected. As a research tool, the reflex has been evoked to gain an understanding of the underlying neural pathways involved (Dietz and Sinkjaer 2007).

There are several techniques to impose a lengthening of the target muscle. The most recent studies using joint rotation perturbations have applied ramp-and-hold-type stimuli; many other types of stimuli, including tapping the tendon using hand-held devices, feedback control tappers, and sine waves have also been implemented (Beith and Harrison 2004; Burke and Schiller 1976; Frigon et al. 2011; Mrachacz-Kersting et al. 2006; O’Sullivan et al. 1998; Suresh et al. 2005; van Doornik et al. 2009). The response recorded in the target muscle is dependent on the technique used. For example, following a tap to the Achilles tendon, a single synchronous peak occurs (Gandevia et al. 1986), whereas ankle joint rotation will result in several synchronous peaks (Sinkjaer et al. 1988). Other than the peaks in the EMG, there are also silent periods between the peaks that can only be observed if the studied muscle has some background activity preceding the stretch stimulus (Matthews 1984; Miles et al. 1995; Poliakov and Miles 1994).
In the brain slice experiments, another method was also tested that has been claimed to overcome the autocorrelation/synchronization-related errors: peristimulus frequencygram (PSF; Türker and Cheng 1994). The basic theory that underlies the PSF method stands on the assumption that there is a positive linear relationship between input current into a motoneuron and its discharge rate (Powers et al. 1992). The PSF method simply plots the instantaneous discharge rates of single motor units against the time of the stimulus. The brain slice experiments have demonstrated that the time course of the net input current to a motoneuron is more accurately illustrated using the PSF method compared with the probabilistic methods (Türker and Powers 1999, 2003, 2005).

The aim of this work was to use both the probabilistic (SEMG and PSTH) and discharge rate (PSF)-based methods to reinvestigate the time course of the postsynaptic potential in human tibialis anterior (TA) motoneurons induced by various stretch stimuli. We expect that some of the reflex responses “established” using probabilistic methods might be synchronization-related artifacts. We have also examined different stimulation methods of inducing stretch reflexes to test that our comparison of analysis methods would be applicable to various forms of inducing stretch reflexes in human subjects and also to test the claim that the different forms of inducing stretch reflex may initiate varying number of reflex responses (Gandevia et al. 1986; Sinkjaer et al. 1988). First, we hypothesize that the shape and the speed of the stimulus will determine the properties of the reflex responses. Second, we propose that the PSF analysis will identify different numbers of reflex responses compared with the probabilistic analyses.

METHODS

The experiments were approved by the Human Ethics Committee of the University of Aalborg (N-20090019), and the participants gave written informed consent according to the Declaration of Helsinki. The reflex responses of 107 low-threshold (<20% maximal voluntary contraction; 4–19% as a range of maximum EMG and 12–38% as a range of maximum force) TA motor units were recorded in 12 volunteers (age 31 ± 5 yr; range 25–40 yr). The SEMG and potentials from single TA motor units (SMU) and the force from the footplate were recorded on a computer using the CED interface (Cambridge Electronic Design, Cambridge, UK) for off-line analysis. The SMUs were recruited by isometric contraction of the muscle as the foot was dorsiflexed against a foot pedal, which was attached to a force transducer. Subjects contracted their muscles to activate one or two motor units on the single-unit electrode. They were provided with the feedback of the activity of the most prominent unit with the largest motor unit action potential and asked to keep the discharge rate of the selected unit at a rate of ~6 Hz using the sound of the accepted unit pulses. While the subject maintained the background level of excitation of the muscle’s motoneuron pool at a constant level, stretch stimuli were applied and reflex responses of simultaneously recorded surface EMG and SMUs were recorded. Briefly, the procedures outlined below were used.

Recordings

Surface EMG. Surface EMG of the right TA was recorded using bipolar electrodes placed 2 cm apart according to the recommendations of Garland et al. (1994). The skin overlying the muscle was cleaned using 70% alcohol to reduce electrode resistance to below 5 kΩ. The signals were amplified 1,000 times, bandpass filtered with a cutoff frequency of 20–500 Hz, and recorded on the CED system. Data were analyzed using the software available in the CED Spike2 data acquisition system.

Single motor units. To record motor unit activity, three pairs of Teflon-insulated silver wires (75 μm in core diameter) were inserted into the muscle with 25-gauge needles; the needles were then withdrawn, leaving the wires in the belly of the muscle. In most experiments, stable recordings of one or two units were obtained from each of the insertion sites. The shape of the SMU action potential was discriminated online using a template-matching algorithm (version 6; Spike2), which generated a recognition pulse whenever it matched the shape of an action potential. Single-unit potentials were sampled at 20,000 Hz and recorded on a computer for off-line analyses (data acquisition and analysis system, CED Spike2).

Stretch Stimulus

The right leg was fastened to a servo-controlled hydraulic actuator (model 215.35; MTS Systems) (Voigt et al. 1999). The angle of the ankle was arranged to the anatomical rest position of the subject, and the subject chair was arranged to extend the knee angle to 120°. The foot segment of the right leg of the subject was firmly strapped to a custom-made plate that extended from the actuator, thus producing a tight interface between the arm of the motor and the foot of the subject, ensuring that the movement of the actuator was transmitted solely to the ankle joint (Fig. 1). Also, the subject’s thigh was strapped to the chair to ensure that movement of the actuator did not change the knee and hip angle. The angular position of the actuator was monitored by an angular displacement transducer and feedback controlled using a proportional integral derivative (PID) control system (DC ADT series 600; Transtek). Stretches were randomly applied at intervals ranging between 5 and 7 s. Three different types of perturbations were imposed randomly for each subject: a tap, a ramp-and-hold, and a half-sine perturbation (Fig. 2). The angular velocity, amplitude, and duration were 100°/s, 1°, and 0 ms for tap perturbations and 200°/s, 4°, and 460 ms for ramp-and-hold perturbations; amplitude and duration were 1° and 100 ms for half-sine perturbations.

Analyses

Surface EMG. Raw SEMG records were full-wave rectified and averaged 500 ms before and 250 ms after the imposed perturbation, spanning an analysis time of 750 ms. Cumulative sum (CUSUM; Ellaway 1978) was calculated from the averaged SEMG to illustrate the exact timing and the size of the reflex response. Any poststimulus deflection that is larger than the maximal prestimulus deflections (error box) and appears before the reaction time to the perturbations was considered a genuine (significant) reflex response to the stimulus (Brinkworth and Türker 2003; Türker et al. 1997).

Single motor units. Single motor units were identified using the Spike2 program (CED), and each unit was separated using the program’s template-matching algorithm. PSTHs and PSFs were then constructed from the times of occurrence of action potentials of individual motor units. To build the PSTH, firing times of each selected unit were converted into acceptance pulses by the Spike2 program and placed into 1-ms bins around the time of the perturbation stimulus. Once acceptance pulses belonging to an SMU are placed in these bins during 100 or more triggers, the end product, the PSTH of that unit, indicates the number of occurrences of spikes at each of the bins around the time of the stimulus. PSF is the superimposition of the instantaneous discharge rates of a selected unit around the time of the stimulus and indicates the excitability of the motoneuron membrane (Powers and Türker 2010; Türker and Powers 2005). To build the PSF, acceptance pulses from Spike2 program were turned into instantaneous discharge rate values. Instantaneous discharge rate values obtained using 100 or more triggers were superimposed around the time of the stimulus.

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CUSUMs for the PSTH and PSF graphs were also obtained to make any subtle but persistent changes detectable. CUSUM of the PSF highlights the changes in the discharge rate after the stimulus by adding the differences in the bin values from the average prestimulus bin value (Türker et al. 1996). This is essentially the same approach as the original CUSUM that was built for the PSTH (Ellaway 1978). The only difference is that the CUSUM of the PSTH uses the number of counts in each bin, whereas the CUSUM of the PSF uses average discharge rate values in each bin.

The reflex responses of one or two units were determined from each electrode off-line. The chosen units were always the ones with the largest action potentials because they were easier to identify and follow online during the experiment. The motor unit potentials were high-pass filtered at around 1,000 Hz to further clarify the shape and amplitude of the unit of interest and to depress the amplitude of all other units including low-frequency noise in the record. This process reduces the chances of superimposition of other unit potentials onto the action potential of the unit of interest. Such superimpositions can interfere with the recognition of the unit potential by the template-matching program and hence undermine the estimation of the reflex response. Other than using filtering techniques to highlight the unit of interest clearly, we have also used two other approaches in this experiment so that recognition of the unit throughout the experiment would be possible. These were the limitation imposed on the

![Fig. 1. Experimental setup. A schematic view shows the foot-actuator interface and the forces and accelerations measured. The position of the adapter was adjusted such that the anatomic axis of the ankle joint of each subject coincided with the turning point of the actuator (TP). The imposed forces were measured with a linear load cell (Slimline; Kistler) attached to the footplate. The acceleration was measured with an accelerometer (KSHEAR Piezotron; Kistler) attached in line with the load cell. Amp Ch1 and Amp Ch2, amplifier channels 1 and 2.](image)

![Fig. 2. Joint angle changes and reflex responses. The motor unit responses to 3 different perturbations (tap-like, A; ramp and hold, B; half-sine, C) are shown. Dashed vertical lines indicate the trigger time of perturbation. Top recordings show the change in joint angle during perturbations; middle and bottom recordings show the cumulative sums (CUSUM) of peristimulus time histogram (PSTH) and peristimulus frequencygram (PSF) analyses.](image)
stimulus intensity and the number of units in a given intramuscular electrode. In an ideal setting, the electrode contained only one motor unit with a large signal-to-noise ratio and the stimulus intensity did not recruit other (previously nonactive) units at the reflex latency. If these two conditions were met, reliable and repeatable results were obtained.

Following these somewhat limiting preconditions, the amplitude of the imposed perturbation was fixed to 1° for the tap stimulus, 4° for the ramp-and-hold stimulus, and 1° for the half-sine stimulus. These amplitudes were used because they recruited at least 2 spikes at the onset of the short-latency response to the imposed stretch in 10 consecutive stimuli. Usually, our aim was 2–4 spikes at the reflex latency (out of 10 stimuli), equivalent to an excitatory postsynaptic potential (EPSP) size of about 2–4 mV (Ashby and Zilm 1978; Miles et al. 1989), while setting the perturbation amplitude in an experiment. Any further increase in the perturbation amplitude often recruited extra motor units at the stretch reflex latency and hence interfered with the recognition of the selected motor unit at this latency. High contraction levels also could not be studied because they often recruit many more motor units, which are also reflected in the intramuscular electrode recordings. When an intramuscular EMG record has many units, the stimulus intensity that can induce stretch reflexes in SEMG would induce many superimpositions of motor unit potentials at the reflex latency and hence would not allow for an accurate study of the PSPs to single motoneurons.

At this level of intensity, several hundred stimuli were delivered to quantify the net response to the stimulus. To achieve this objective in this study, the stimuli continued to be delivered as long as the shape of the motor unit potentials was distinctly recognized and for as long as the subject was able to fire the unit regularly. The units that were lost before delivering at least 100 stimuli were not analyzed further. More than 100 stimuli (279 ± 166; range 100–986) were delivered for each of the 107 units reported in this article. Since the number of stimuli varied between the units, we normalized the size of the reflex responses to the number of triggers used. Hence we obtained values such as extra spikes per trigger or extra Hz per trigger (see Tables 1–3). Latencies were determined from the turning point of any of the significant deflections in the CUSUM records. Amplitudes of the reflex responses were determined using the method described by Brinkworth and Türker (2003). In essence, the reflex response was normalized to the maximum possible inhibition (no active units for the entire duration of the reflex) and marked as the percentage of this maximum inhibition.

Statistical analyses. The latencies of responses between analysis techniques were investigated using one-way ANOVA. The differences between each group were tested using Fisher’s least significant difference post hoc test. Also, the amplitude of responses between tap-like and ramp-and-hold stimulation types was investigated using one-way ANOVA. The incidence rates of responses were compared between SEMG, PSTH, and PSF methods for each stimulation method (tap, ramp and hold) using the McNemar statistical test. The prevalence values of M1, M2, and M3 for different SMUs were grouped according to their prestimulus average discharge rates into three groups as low (6–8 Hz), middle (8–10 Hz), and high (10–12 Hz) discharge rate. The differences between these groups were compared using the \( \chi^2 \) statistic test, statistical test for the PSTH and PSF analysis methods. For all statistical analyses, the null hypothesis was rejected if probability was <0.05.

RESULTS

Reflex responses to a minimum of 100 and a maximum of 986 imposed perturbations of 107 units were analyzed. Three types of stretch stimuli were applied, and results were analyzed using three different methods. The stimuli were simple tap-like stretch, ramp-and-hold stretch, and half-sine stretch. Stimulus-induced responses in SEMG and SMUs were analyzed using the classical probability-based technique, which involved building rectified averaged SEMG PSTHs at the amplitude of the stimulus and constructing PSTHs from the firing times of the single units. The same SMU data were also analyzed using PSFs from the instantaneous discharge rates of the units.

Simple Tap-Like Stretch Stimulus/Perturbation

Tap-like stimulus was applied to 12 subjects, and 72 motor units were recorded. Using PSTH as the indicator, we found that the tap-like stretch stimulus induced five separate significant responses: the first response was a short-latency excitatory response (M1), or the short-latency stretch reflex; the second was a silent period (SP1); the third was a medium-latency excitation, or M2; the fourth was a long-latency silent period (SP2); and the fifth was a broad peak (M3) (Table 1 and Fig. 3). With the use of the same motor unit data, the PSF technique indicated that the tap-like perturbation induced only three significant reflex responses corresponding with the M1, M2, and M3 responses on the SEMG and PSTH (Table 1). The PSF displayed significant increases in the discharge rate of the unit during the first excitatory reflex in the PSTH. Although the second (M2) and the third (M3) responses were rarely observed in the same record (10 in PSF and 18 in PSTH over 72 motor units), when they did occur the discharge rate of the unit underlying these responses increased significantly. On the other hand, discharge rate did not decrease significantly during either SP1 or SP2 (Table 1), hence suggesting that these silent periods do not indicate inhibitions.

Ramp-and-Hold Stretch Stimulus

In 16 units in 11 subjects, ramp-and-hold stretch stimuli/perturbations were applied. In all experiments, this type of stimulus induced several peaks and troughs in the SEMG and PSTH similar to the responses to the tap-like stretch stimuli. PSF illustrated genuine increases in the discharge rates underlying most of the peaks in the SEMG and PSTH (Fig. 4). Again, there was no significant decrease in discharge rate underlying the silent periods that were observed in SEMG and PSTH records. Latencies and amplitudes of the reflex responses are given in Table 2.

Half-Sine Stretch Stimulus

Half-sine stimulus was applied to 8 subjects, and 19 motor units were recorded from those subjects. These stimuli induced a long-lasting excitation (LLE) followed by a long-lasting silent period (LLSP) in SEMG and PSTH. The increase in the discharge rate (as displayed in the PSF records), however, lasted for the entire duration of the mechanical stimulus and continued even during the phase where the number of spikes decreased in the SEMG and PSTH (Fig. 5). Table 3 details the latencies and amplitudes of the reflex responses elicited using half-sine stretch stimuli.

Comparison of Latency, Duration, and Amplitude of Reflex Responses

No differences were found between the latencies of the M1 responses determined using the two different analysis methods. However, the response amplitudes that were induced by ramp-
M1 47 ± 3 15 ± 2 73 ± 23 67/72
M2 76 ± 3 10 ± 3 44 ± 22 23/72
M3 131 ± 20 42 ± 16 25 ± 9 57/72
S1 62 ± 3 27 ± 14 39 ± 15 60/72
S2 89 ± 19 38 ± 19 29 ± 13 38/72

Table 2. Latencies, durations, and amplitudes of significant deflections (reflex responses) in CUSUMs of SEMG, PSTH, and PSF for ramp-and-hold stimulus

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M1 48 ± 3 19 ± 4 83 ± 42 15/16
M2 81 ± 6 20 ± 9 86 ± 52 14/16
M3 131 ± 17 40 ± 13 35 ± 19 12/16
S1 67 ± 4 13 ± 2 30 ± 8 8/16
S2 100 ± 10 37 ± 14 42 ± 20 13/16

Table 3. Latencies and amplitudes of significant deflections (reflex responses) in CUSUMs of SEMG, PSTH, and PSF for half-sine stimulus

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LLE 68 ± 13 36 ± 16
LLSP 145 ± 1 21 ± 2

Comparison of the Number of Reflex Responses

The responses to the tap-like stretch and the ramp-and-hold stretch stimuli often generated similar numbers of reflex responses. PSF analyses indicated that only the M1, M2, and M3 responses on the SEMG and PSTH were genuine excitatory events, since the discharge rate of single units significantly increased during these periods. The differences between incidence rates of excitatory responses in each of the analysis methods were quantified using the McNemar test. The first excitatory response was observed in almost every motor unit for each method (for tap-like stretch: SEMG 93%, PSTH 88%; PSF 83%; for ramp-and-hold stretch: SEMG 93%, PSTH 88%, PSF 88%). For the tap-like stimulus, the incidence rate of M2 in PSF (67%) was significantly higher than that in SEMG (32%) and PSTH (37%) ($P < 0.05$ for both pairs). In contrast with M2, the incidence rate of M3 was significantly higher in SEMG (80%) and PSTH (68%) compared with that in PSF (30%) ($P < 0.05$ for both comparisons; Table 1). Thus the incidence of reflex responses was one of the differences between the probability- and discharge rate-based analysis methods. The other major difference between the methods was the incidence of the silent period responses. The tap-like and
ramp-and-hold stretch stimuli induced silent periods that followed the M1 and M2 responses in the classical analysis methods, whereas no such periods were observed in the PSF technique (Fig. 6).

The prevalence of the M1 and M2 responses depended on the discharge rate of the units. As the background discharge rate increased, the prevalence of the M2 responses also increased, whereas the incidence of the M1 response decreased, in the PSTH records (Fig. 7). The PSF records also indicated an increase in the prevalence of the M2 response with the increase in the background discharge rate of the unit. The amplitudes of these responses were also related to the background discharge rate of the underlying units.

The significance of the difference between the three discharge groups (low, medium, and high) for the prevalence values for M1, M2, and M3 was compared using the \( \chi^2 \) test. The null hypothesis was that the prevalence does not depend on the discharge rate of units (\( \chi^2_T = 5.990 \) for 2 degrees of freedom). For the PSTH method, no significant differences were found for the prevalence values of M1 and M3 for any of the three different discharge rates (M1: \( P > 0.05, \chi^2_H = 0.293; \) M3: \( P > 0.05, \chi^2_H = 1.659 \)). However, a significant difference was found for the prevalence values of M2. For the PSF method, no significant differences were found for the prevalence values of M1 among groups (\( P > 0.05, \chi^2_H = 0.869 \)). On the other hand, the prevalence values for M2 and M3 were significantly different among at least one pair of discharge rate groups (M2: \( P < 0.05, \chi^2_H = 5.996; \) M3: \( P < 0.05, \chi^2_H = 7.539 \)).

In one subject, the tap-like stretch stimulus induced only M2 in one unit, whereas another simultaneously recorded unit displayed both M1 and M2 responses. This was a rare event and was only observed in 1 of 72 units tested using this type of stretch stimulus.

**DISCUSSION**

There are two main new findings in this study. First, rejecting our first hypothesis, this study has shown that the tap-like stretch induced a similar number of reflex responses as the ramp-and-hold type stretch stimulus. Second, supporting our second hypothesis, whereas probabilistic analyses indicated several peaks and troughs in the records, discharge rate-based methods only confirmed the sign of excitatory responses.

**Analysis Methods**

In this study, we have used three methods to determine the reflex responses: rectified and averaged SEMG, PSTH, and PSF. The SEMG and PSTH methods are both conventional
methods that have been used in reflex studies for ~35 years. Both methods illustrate the density of motor unit spikes around the time of stimulation (Ellaway 1978). With these methods, poststimulus periods with spike densities lower than those during prestimulus periods are labeled as inhibition, and those with higher densities are labeled as excitation (Brooke et al. 1999; Okdeh et al. 1999; Sonnenborg et al. 2000). However, use of only these classical methods gives an incomplete picture of the synaptic connection for the following reasons. First, they rely on the number of motor unit spikes occurring at a fixed time from the stimulus. This number may be misleading if part of the reflex response falls in the shadow of an earlier and more powerful reflex response (Awiszus et al. 1991; Türker and Cheng 1994). Second, a strong excitatory or inhibitory reflex response can synchronize the timing of the action potentials in relation to the stimulus. This synchronous discharge can induce several peaks and troughs in both SEMG and PSTH as the motoneuron discharges at a regular rate after the initial reflex response (Awiszus et al. 1991; Miles and Türker 1987; Türker and Cheng 1994). However, the PSF represents the instantaneous discharge rate of the motoneurons (Türker and Cheng 1994). Because the discharge rate of a motoneuron directly represents the effective net current injected into it either slowly (Granit et al. 1963; Powers et al. 1992) or rapidly (Baldissera et al. 1982), any significant change in the discharge rate of a motoneuron as a result of a stimulus can be used to estimate the

Fig. 4. Reflex response of a single TA motor unit to the ramp-and-hold stretch stimulus (n = 204 stimuli). Properties of the traces, horizontal lines, and vertical lines 1–6 are similar to those indicated in Fig. 3. Again, whereas SEMG and PSTH indicated at least 4 significant reflex responses, PSF only confirmed the 2 excitatory events, M1 and M2, as genuine reflex responses where the discharge rate of the unit increased significantly.

Fig. 5. Reflex response of a single TA motor unit to the sine-type stretch stimulus (n = 283 stimuli) applied on the Achilles tendon. Properties of the traces, horizontal lines, and vertical lines 1–3 are similar to those indicated in Fig. 3. There were only 2 significant reflex events (LLE, long-lasting excitation; LLSP, long-lasting silent period) in SEMG and PSTH but only 1 (LLE) in PSF.

The CUSUM of the average records is also necessary because it amplifies small but persistent changes (Ellaway 1978). Because CUSUM indicates the area of responses, it can also be used to estimate the relative strength of reflex responses (Binboğa and Türker 2012; Brinkworth and Türker 2003).

**Reflex Responses to Different Shape of Stimulation**

The response recorded in the target muscle is dependent on the technique used (Gandevia et al. 1986; Sinkjaer et al. 1988). Moreover, it is known that depending on the shape, slope, and amplitude of mechanical stimulation, different sensory afferents can be activated (Matthews 1984). An additional complication arises when the same perturbation is applied to either a hand muscle or a lower limb muscle. Typically, in both instances various peaks may be observed, which have been labeled M1 and M2, with an additional peak often seen in lower limb muscles (M3) (Petersen et al. 1998). Therefore, in the present study, three different stimulation shapes (tap, ramp and hold, sinusoidal) were used to investigate the stretch reflex on TA muscle.

The only difference between these methods was the occurrence of silent periods that followed the M1, M2, and the LLE responses in the classical methods. These silent periods with a low number of action potentials were not recognized as periods of inhibitions in the PSF technique, since during these periods the discharge rates of action potentials were the same as during the prestimulus periods. Therefore, it would be wrong to label these periods of low motor unit activity as inhibitory reflex responses. For a period to be labeled as an inhibitory period with the PSF method, the discharge rate of the underlying motor units needs to decrease below the prestimulus discharge rate values (Türker and Powers 1999, 2003, 2005). We suggest that the low number of spikes was possibly caused by the phase advance of spikes as a result of the stretch stimulus. The phase advancement generated the M1 response. The same phase advancement must also have applied to the SP2 period that induced the M2 response. Genuineness of the M2 response is clear because not only was it present in most units but also it became more prominent with the increase in the discharge rate. In the lower discharge rates, it must be underestimated due to the shadowing effect of the M1 response.

For the TA muscle it is known that the M1 component arises from the activation of group Ia muscle afferents, which synapse directly onto the motoneuron (Darton et al. 1985; Grey et al. 2001). The neurological basis of the M2 and M3 response, however, is not so clear and has been the subject of much controversy (Burke et al. 1983; Christensen et al. 2001; Lee and Tatton 1978; Sinkjaer et al. 1999). The origin of M2 in hand muscles has been claimed to be cortical (Matthews et al. 1990), whereas the M2 response in lower limb muscles occurs too fast to be cortical (Darton et al. 1985; Thilmann et al. 1991). Reports have suggested a group Ib or group II pathway may be responsible for the M2 response (Dietz 1998; Dietz et al. 1985; Grey et al. 2001; Schieppati and Nardone 1997). The origin of the M3 response has been claimed to be via a group Ia pathway, which traverses the motor cortex (Petersen et al. 1998). It is also possible that the M2 and M3 responses may be due to the synchronous reoccurrence of spikes after the M1.
Although several studies have deliberated possible pathways for the excitatory responses, only a few studies have reported silent period in the SEMG or PSTH after the M1 response (Matthews 1984; Miles et al. 1995; Poliakov and Miles 1994). Moreover, silent period after the M2 response has never been reported. The reasons for not recognizing the silent periods in earlier work may be twofold. First, most previous stretch reflex studies involved relaxed muscles, which make observing inhibitory responses difficult (Avela et al. 1999; Fellows et al. 1993; Thilmann et al. 1991; Yamamoto et al. 2000). In the present study, the subjects maintained a fixed level of excitation of their muscles to make sure that the excitatory as well as the inhibitory pathways could be identified if they existed. The other possible reason for not recognizing the silent periods, even when the existence of such periods was possible when a low spike density was used as the criterion (as in SEMG and PSTH methods), may have been the fact that most did not use analysis methods that brought out subtle but consistent changes in the SEMG or PSTH, such as the CUSUM technique (Darton et al. 1985; Edin and Vallbo 1990; Grey et al. 2001; Mrachacz-Kersting et al. 2006; Petersen et al. 1998; Schuermans et al. 2009; Thilmann et al. 1991).

In the present study, we also used the sinusoidal stimulation to find evidence of the contribution of group II afferents. It is known that group I and II afferents exhibit different dynamics (Cooper 1961; Matthew 1984; Matthews and Stein 1969). Whereas group II afferents are sensitive to changes in the length of muscle, group I afferents are activated by the acceleration in the force (Edin and Vallbo 1990; Matthews 1984). In this study the slow stretch (sinusoidal) stimulus induced two significant reflex responses in SEMG and PSTH, excitation followed by silent period. However, the PSF analysis has again differed from the probabilistic analyses and indicated only one significant reflex, excitation. The latency of this excitation was longer than the latency of the M1 response that was induced by ramp-and-hold and tap-like stimulations. These findings suggest that slow stretch-induced excitatory response may originate mainly from the slower conducting group II afferents.

**Effect of Discharge Rate on Prevalence of Reflexes**

Prevalence of the M1 and M2 responses depended on the discharge rate of the units underlying these responses. As the background discharge rate increased, the prevalence of the M2 response increased whereas M1 decreased in the PSTH and PSF records. The size of these responses was also related to the background discharge rate of the underlying units. Whereas the M1 response amplitude decreased with the increase in the background rate of the unit, the amplitude of the M2 response actually increased along with the rate. This is an evidence that the slope of the membrane potential trajectory becomes steeper as the discharge rate increases (Ashby and Zilm 1982; Miles and Türker 1987; Miles et al. 1989), hence allowing the appearance of the long-latency PSPs that were hidden behind earlier PSPs at low discharge rates. Because the background discharge rate affected the prevalence of these responses, it is put forward that two responses are definitely genuine and that the M2 response is generally larger than the M1 response but often appears smaller since it falls in the shadow of the earlier M1 response at low contraction levels (SEMG) or low discharge rates (PSTH and PSF).

**An Odd Finding**

In one subject, the tap-like stretch stimulus induced only M2 in one unit, whereas another simultaneously recorded unit responded with both M1 and M2, which were also indicated in the SEMG. This was a rare event and was only observed in 1 of 107 units tested. However, this may indicate that some units are only connected with the spindle primary afferents via a long and/or polysynaptic pathway.
Concluding Remarks

The aim of this work was to use both the probabilistic (SEMG and PSTH) and discharge rate (PSF)-based methods to investigate the time course of the postsynaptic potential in human tibialis anterior motoneurons induced by the stretch stimuli. We showed earlier that the discharge rate of a motoneuron is a better representative of the postsynaptic potential than the classically used probabilistic methods. However, since the current knowledge on the stretch receptor pathways are “established” using only the probabilistic methods, we wanted to analyze the same reflex data using both of these methods so that we could compare the two methods and possibly revise some of the current beliefs. The present study indicates that the neuronal pathways of the stretch reflex may include up to three phases of excitations and exclude inhibitory pathways.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


