Estimating Exercise-Induced Changes in Human Neuronal Networks

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TÜRKER, K. S. Estimating exercise-induced changes in human neuronal networks. *Exerc. Sport Sci. Rev.*, Vol. 49, No. 3, pp. 147–156, 2021. *Although several methods have been used to estimate exercise-induced changes in human neuronal networks, there are growing doubts about the methodologies used. This review describes a single motor unit–based method that minimizes the errors inherent in classical methods. With this method, it is now possible to identify human neuronal networks' changes due to exercise.* **Key Words:** electromyography, reflexes, cortical silent period, motor evoked potential, transcranial magnetic stimulation

Key points

- Several methods have been used to study exercise-induced changes in human neuronal networks.
- These methods attempted to identify changes due to exercise in cortical, corticospinal, and motoneuronal networks related to muscle strength, fatigue, and resilience.
- Using computer simulations and rat brain slice experiments on tonically active motoneurons, we have demonstrated that the classical methods used to obtain these networks' functional connections contain errors.
- These brain slice experiments proposed a motor unit–based peristimulus frequencygram (PSF) method that minimizes the classical systems' errors.
- Several studies have already used the PSF method to reconsider the human nervous system networks' functional connections.
- With the PSF method, it is now possible to study the changes in the human neuronal networks' functional connections due to exercise.

INTRODUCTION

Importance of Movement

The life of a sea squirt can demonstrate one of the most appropriate examples of the importance of movement for the nervous system's health. When born, this little animal resembles a tadpole, with a long tail, simple eye, backbone, slim nerve cord, and a brain. When it is young, the animal has a well-developed nervous system, enabling it to move, balance, feel, and choose and consume food particles as it moves around the sea searching for food. However, after identifying an appropriate location, one with good water current that brings fine food particles, the squirt fixes itself to that position, and from then on stays there. The subsequent lack of movement and the need to actively search for food means that the animal's brain and nervous system disappear entirely within a few weeks (for more details, see https://www.britannica.com/animal/sea-squirt).

Of the various types of human movements, some involve only a few neurons, whereas others involve complex neuronal networks. For example, reflex movements entail only a few neurons and are highly stereotypical; at the opposite end of the spectrum, voluntary movements require several nervous system networks and are highly modifiable. To understand goal-directed voluntary movements' workings, an appreciation of how simple neuronal networks function is required. To take account of the evolution of methods that aimed to study the functional connections of movement-related neuronal networks in the human nervous system, this review will cover several headings:

- Importance of neuronal network studies in exercise sciences.
- Classical methods for studying functional connection of neuronal networks.
- The emergence of methods identifying possible errors in classical methodologies and proposing the peristimulus frequencygram (PSF) method that minimizes such errors.
- Reconsidering functional connections of the human neuronal networks using the PSF method.
- Future perspectives combining the PSF method with the noninvasive surface electromyography (EMG)–based motor unit recording techniques.

IMPORTANCE OF NEURONAL NETWORK STUDIES IN EXERCISE SCIENCES

The neuronal structures connecting peripheral receptors and motor cortex to lower motoneurons are defined as "neuronal networks" in this review. Excitability modulations of these networks

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have been routinely used in the exercise sciences. Although hundreds of studies have addressed this topic, this review will mention only the most recent and most directly relevant examples. We should warn the readers that the examples given here are only a small portion of the work in this field and that the findings are highly dependent on the task involved.

Changes in the Cortical and Corticospinal Networks' Excitability During or Immediately After Exercise Training

To assess these pathways' excitability, single or double pulse transcranial magnetic stimulation (TMS) is delivered to the motor cortex, and stimulus-induced EMG responses of voluntarily active muscles are analyzed. Suprathreshold TMS stimulus induces a direct motor response (the so-called "motor-evoked potential" or "MEP") followed by a period of reduced EMG activity (the so-called "cortical silent period" or "CSP"). CSP has been claimed to be appropriate for investigating the inhibitory effects of cortical and corticospinal control of the voluntary motor output. On the other hand, the MEP amplitude has been claimed to correlate with the cortical and spinal motoneuron excitability directly and used as an index for excitatory motor pathways' stability (reviewed in (1)).

It has been shown that fatiguing cycling exercise that involves several limb muscles reduces muscles' ability to generate force and the motor cortex's capacity to recruit muscles fully (2). In this study, the lack of change in MEP amplitude and CSP duration suggested that the corticospinal pathway's responsiveness was not modulated during these fatiguing exercises. However, these findings contrast to the findings in single-limb fatiguing exercises. In these studies, it has been found that the cortical excitability increases, the duration of CSP decreases, and the MEP response increases (3).

Working on the effect of high-intensity training of the less-affected arm of patients who have had a stroke, Sun *et al.* (4) reported decreased CSP duration from both hemispheres. These findings suggested that training the neurologically less affected arm can improve strength bilaterally and alter spinal and cortical excitability (4). Working on the fatigue levels of patients with multiple sclerosis, Chaves *et al.* (5) found that the CSP duration could indicate these patients' fatigue levels, that is, the longer the CSP, the more significant the fatigue. Opplert *et al.* (6) worked on the effect of pre-exercise static stretch and found that the corticospinal excitability increased after static stretch.

Changes in Spinal Networks' Excitability Because of Exercise

Excitability of spinal networks has been assessed using the tendon reflex, H-reflex, and the duration of a silent period (SP) that follows these reflexes. Spinal excitability indicated by the H-reflex size did not change after pre-exercise static stretch (6). Downslope walking has been found to induce depression in the H-reflex size (7). Five minutes of static stretch has been found to decrease the tendon reflex but increase the H-reflex (8). H-reflex size has been found to increase immediately after full-body resistance exercise (9). Movement-related afferent inputs have been found to firmly depress the H-reflex in the soleus muscle during walking (10). Research by Christensen *et al.* (11) found that exhaustive maximal teeth clenching increased the monosynaptic jaw jerk (tendon) reflex's SP duration by about 35%.

Changes in Motoneurons' Excitability During Movement and Exercise

Motoneuron excitability has been studied using single motor unit discharge characteristics. There is a one-to-one relation between a motoneuron's discharge and the recording of a single motor unit action potential (SMU-AP). Using SMU-APs, it has been found that the motor unit discharge behavior is altered as a function of exercise training status (12,13). In a related study, it has been found that the SMU-AP amplitude is correlated with muscular strength and power (14). After finding that the larger motor units recruit at high-intensity contractions compared with fatiguing moderate-intensity contractions, it has been suggested that individuals seeking to generate maximal activation of their motor pools should use high-intensity exercise training rather than moderate-intensity fatiguing contractions (15). Another study found 8 wk of resistance training increased strength and muscle cross-sectional area but not motor unit discharge rate (16).

Changes in the Excitability of the Networks That Connect Cutaneous Receptors to Motoneurons During Exercise

Comparing the cutaneous reflexes of normal individuals with that of patients with chronic ankle instability (CAI) during walking, Madsen *et al.* (17) showed that people with CAI lack a protective unloading reflex response in the triceps surae muscles after cutaneous stimulation during the early stance phase of the gait cycle. They suggested that evaluating cutaneous reflex modulations may help identify neural alterations in the reflex pathways contributing to functional deficits in those with CAI (17). Sasada *et al.* (18) have examined cutaneous reflexes in various arm muscles during leg pedaling and found that the reflex was significantly increased in the flexor carpi radialis and posterior deltoid and significantly decreased in biceps brachii muscles. Based on these findings, they thought their results indicate a link between the legs' rhythm-generating system and the upper limbs' cutaneous reflex pathways (18).

As the above references demonstrate, changes in the neuronal networks have been used in several studies to test the effect of exercise training. These changes are then used to assess the level of improvement in fitness, fatigue resistance, resilience, and endurance of individuals due to exercise. However, because all previous studies on the effect of exercise on the neuronal networks have used the classical methods, these studies need to be reconsidered using the motor unit–based PSF method. We hope that the wide use of PSF method will set the standards for these networks' functional connection to be reliably used to assess the effect of exercise training.

CLASSICAL METHODS FOR STUDYING FUNCTIONAL CONNECTION OF NEURONAL NETWORKS

Neuronal network studies involve stimulation of type-identified receptors in the skin or the neurons in the motor cortex (input to the system) and recording stimulus-induced responses from an individual or a group of muscles (output of the system). Suppose the nervous system is represented as a black box. In that case, a stimulus delivers an exploratory input into the black

Figure 1. Schema of the method used to estimate neuronal networks in human subjects. To study the structure and function of neuronal networks in the nervous system (black box), it is necessary to insert input into the black box and record the stimulus-induced responses from muscles using electrical muscle activity (EMG). Stimulus-induced response from a muscle group can also be recorded as a system's net force/torque output. Net force/torque output is contributed not merely by the muscle under study but from several synergists and antagonists at the same time, as it is not possible to activate only one muscle without at the same time exciting other muscles via either receptor or motor cortex stimulation. Therefore, although the usage of net force/torque output of the system as a result of strong stimuli gives us some idea about the change in the strength of the entire limb muscles due to exercise, it cannot provide us with the exact site of modulation in the neural networks.

box, with an electrical or mechanical recording from a muscle or a limb illustrating the nervous system's response to the stimulus. Therefore, using this input/output method, it is possible to gather some indirect evidence on the neuronal networks' functional connection.

The idea here is to estimate the functional connection of the neuronal networks operating between stimulated receptors and the motoneurons that supply the muscle of interest. Once the functional connection of the neuronal networks has been established, they can be used as scientific foundations for developing techniques to assess exercise training success.

The most appropriate way to study the neuronal networks' functional connection is to use intracellular electrodes in the nervous system. This approach can only be applied in experimental animals by ablating or stimulating certain parts of the nervous system and observing the consequence of the interference by recording directly from the neurons innervated by the network. Although these experiments can elicit some valuable information on the basic structure of neuronal networks, they cannot provide reliable information on the functional connection of the networks, as the experiments are performed on reduced animal preparations (certain parts of the nervous system ablated, decerebrated, or anesthetized) (19,20). These reduced animal preparations also lack the higher-level (supraspinal) inputs that generally work with the nervous system's lower parts (21). Therefore, the functions associated with neuronal networks obtained from reduced animal preparations may be misleading.

Given that direct experiments to obtain neuronal networks' functional connection cannot be performed on human subjects, various indirect methodologies have been established. As shown in Figure 1, the input/output approach is the most common method for studying human subjects' neuronal networks. In these studies, a group of receptors or the motor cortex is mechanically, electrically, or magnetically stimulated, and stimulus-induced responses were recorded from a single or a group of muscles using EMG electrodes or force transducers. However, because these techniques are indirect, they are open to numerous methodological errors in both the stimulation and the recording processes.

The most used method for estimating neuronal networks' functional connection relies on examining stimulus-evoked changes in surface electromyography (SEMG). Several methods have been developed to analyze stimulus-induced changes in SEMG to enable the network's functional connections to be estimated (22). As shown in Figure 2, SEMG needs to be rectified and averaged to estimate the network's functional connections. The cumulative sum (CUSUM; (24–26)) of the rectified + averaged SEMG makes the analysis easy to interpret. Using the peaks and dips in the CUSUM record, claims can be made on the number, sign, and length of the pathways that connect the stimulus site to the motoneuron (Fig. 3).

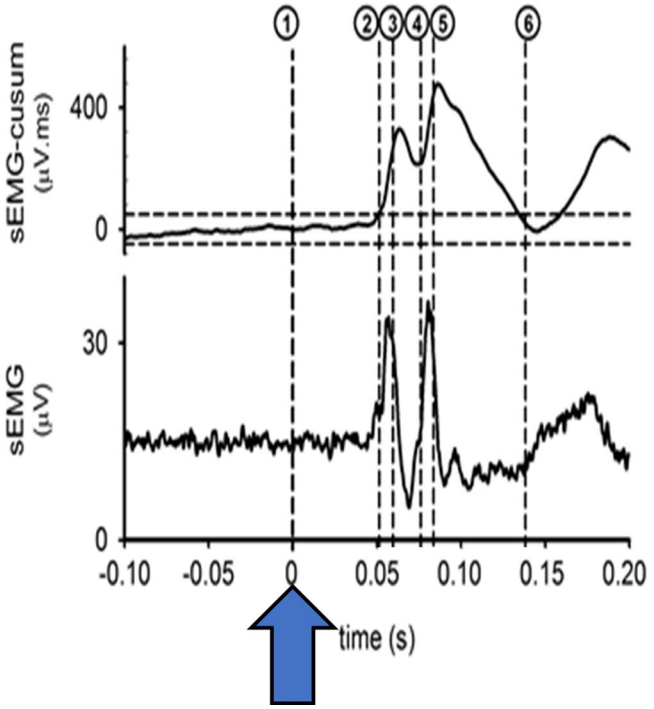

Figure 2. SEMG method for estimating neuronal networks' functional connections: tibialis anterior muscle response to a stretch stimulus. The stimulus is delivered at time zero (vertical arrow: 1). The stimulus-induced response of the tibialis anterior muscle is recorded using SEMG electrodes. The rectified + averaged SEMG (bottom trace) shows two peaks and several troughs, which indicates the complexity of the networks between the muscle spindle receptors and the muscle. The CUSUM (top trace) indicates the peaks and troughs. It has been claimed that each peak flags an excitatory network, and each trough an inhibitory circuitry, which means that five separate networks, each connecting the muscle spindle receptor to the anterior tibialis muscle, need to be described. Each of these networks is shown using vertical dashed lines (2–6). These networks are early excitation (2), early inhibition (3), late excitation (4), late inhibition (5), and extra late excitation (6). Researchers need to identify each of these networks using complex circuitries, as shown in Figure 3. Adapted with permission from The American Physiological Society from Yavuz ŞU, et al (23). Copyright © 2014 The American Physiological Society. All permission requests for this image should be made to the copyright holder.

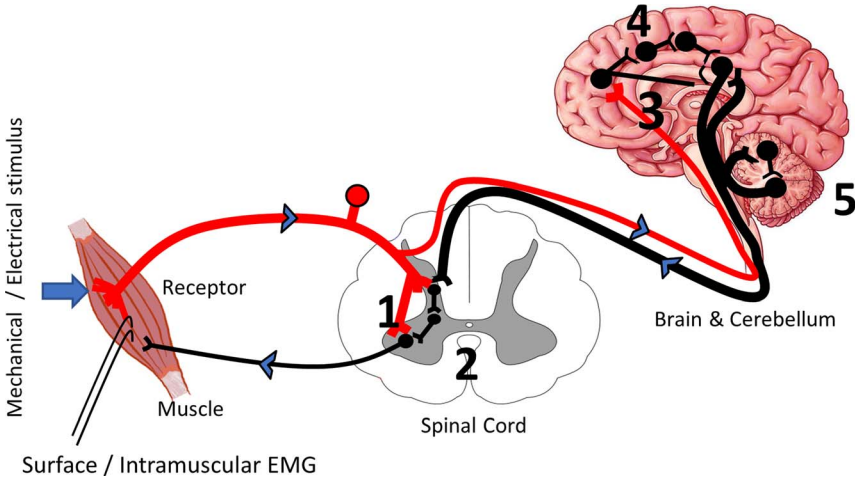

Figure 3. Schema to illustrate the networks that had to be claimed when SEMG is used for the estimation and gave the result illustrated in Figure 2. As initial excitation has a short latency, it can be via the spinal cord (1). In addition, early inhibition has a short latency, indicating a spinal circuit (2). However, late excitation, late inhibition, and extra-late excitation have long latencies; hence, they need to involve longer loops, possibly going through the cerebral cortex and cerebellar nuclei (3–5).

The second most common method for estimating neuronal networks' functional connection is the single motor unit technique. In this technique, intramuscular wire/needle electrodes are inserted into the muscle of interest and the subject is asked to contract the muscle gently. When the subject begins to contract the muscle, the SMU-APs can be observed on a monitor. Because each action potential recorded in the muscle arises directly from a motoneuron in the spinal cord/brain stem, more sophisticated experiments can be performed using the single motor unit recording technique on the functional connection of networks that involve motoneurons. This approach is almost equal to inserting a recording electrode into a motoneuron and studying its function as it usually occurs in the spinal cord. Using the single motor unit technique, as well as illustrating the functional connections of the neuronal network connecting the stimulating site and the motoneuron, it also is possible to estimate neuron's afterhyperpolarization duration, synaptic noise, and trajectory shape (27,28).

The stimulus-induced responses of SMU-AP are often assessed by compiling a peristimulus time histogram (PSTH) and its CUSUM, which measures the probability of occurrence of a motoneuron spike as a measure of time from the stimulus (29) (Fig. 4).

EMERGENCE OF METHODS IDENTIFYING POSSIBLE ERRORS IN CLASSICAL METHODOLOGIES AND PROPOSING A METHOD THAT MINIMIZES SUCH ERRORS

The limitations of the probabilistic techniques (rectified + averaged SEMG and PSTH) were recognized as early as 1970 (30), in that the functional connections of the neuronal networks obtained using the probabilistic analyses depend not only on the characteristics of the underlying synaptic potentials but also on the discharge characteristics of the postsynaptic cell (31–33). When SMU-AP spikes move forward because of an excitatory postsynaptic potential (EPSP), it generates a peak followed by a period where spike numbers decrease. Similarly, when SMU-AP spikes are delayed due to an inhibitory postsynaptic potential (IPSP), it generates a hole followed by a peak (Figs. 5, 6).

To reduce the errors embedded in the probability-based analyses, we and others have proposed methods that use stimulus-induced changes in the interspike intervals and discharge rates of SMU-APs to estimate the functional connections of the neuronal networks produced by stimulation (31–35). One of these methods, the PSF, superimposes stimulus-induced discharge rates to identify networks' functional connections (32,33).

For the primary range of discharges, it has been established that the discharge rate of a motoneuron reflects the net current reaching the soma (36,37). Therefore, any significant change in the poststimulus discharge rate reveals the sign and the time course (profile) of the current injected into the motoneuron due to the stimulus. The injected current's profile reveals the functional connection of neuronal networks connecting the stimulated site with the motoneuron (32,33) (Figs. 3, 7).

To directly test the hypothesis that the discharge rate values identified through the PSF are not affected by previous activity at any time, and hence should be free from the synchronization and count-related errors associated with SEMG and PSTH measurements, we performed experiments on tonically discharging motoneurons in rat brain slices (Fig. 7).

The results demonstrated two issues: firstly, the probability-based analysis methods contain significant errors and cannot be relied on to signify the functional connection of neuronal networks; and, secondly, the PSF reduced the embedded errors associated with the probabilistic analysis and therefore indicated the functional connection of networks more reliably (38–40). The PSF has an additional advantage in that the synaptic input sign is directly reflected in the stimulus-evoked changes in discharge rate. Our results show that, for excitatory inputs, the PSF followed the entire EPSP profile during the first interspike interval. We have reported similar findings for the IPSPs and complex postsynaptic potentials (PSPs) where IPSP and EPSP occur together (38). These studies have clearly shown that the PSF method must be used to study the neuronal networks' functional connection. In the PSF method, an increase in the motor unit discharge rate indicates that the stimulated site has an excitatory neuronal connection with the motoneuron that innervates the single motor unit concerned. A stimulus-induced decrease in the discharge rate, on the other hand, indicates the existence of an inhibitory pathway. Furthermore, the delay between stimulation and the rate change initiation indicates the neuronal pathway's length.

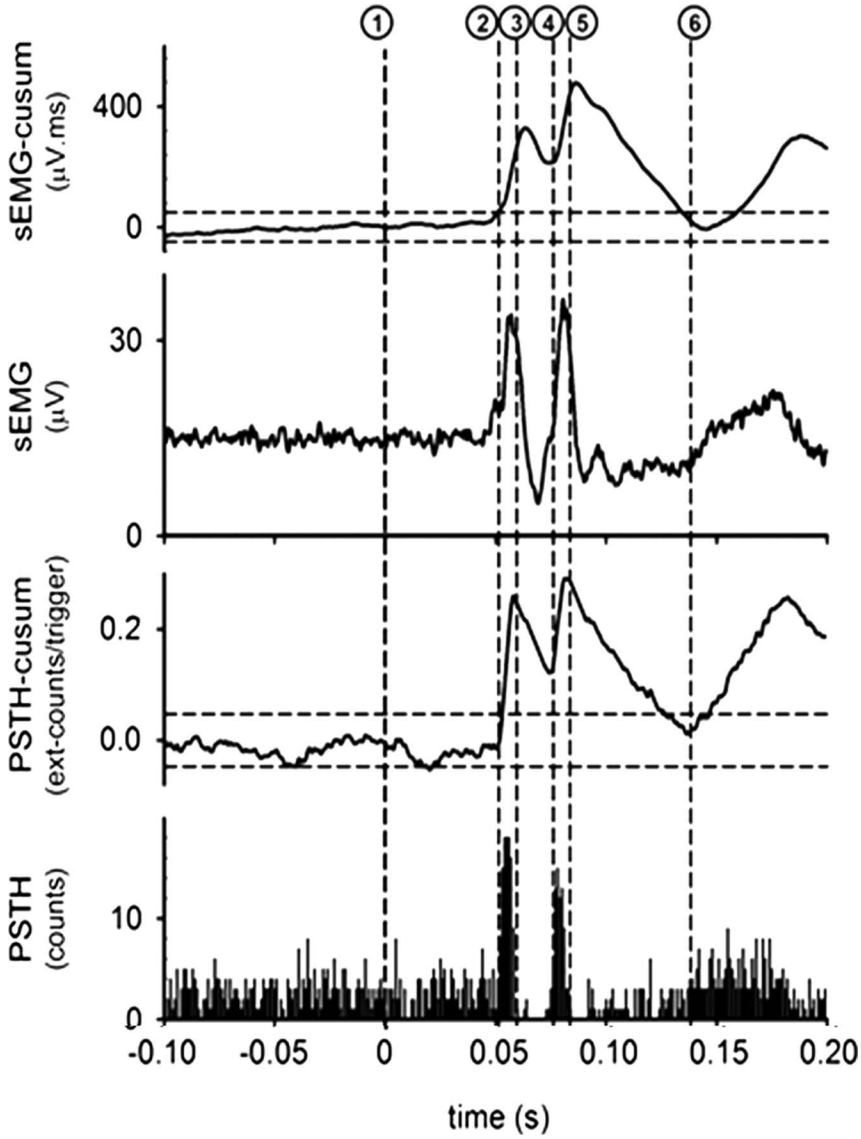

Figure 4. Common probability-based analysis: SEMG and single motor unit PSTH. Tibialis anterior muscle response to a stretch stimulus. Extension of Figure 2. Probability-based analyses rely on several SMU-AP occurrences relative to the stimulus. The PSTH and its CUSUM (bottom two traces) indicate that the stimulus generated five significant events, as shown in Figure 2. Here again, each peak has been considered to represent an excitatory pathway, and each trough an inhibitory neuronal circuit. To make the comparison clearly, we placed simultaneously recorded SEMG and its CUSUM on the top of the PSTH and its CUSUM. Accordingly, the single motor unit analysis also indicates five separate networks, each of which connects the stimulated receptor to the motoneuron in the tibialis anterior motor pool. Therefore, researchers who obtain a PSTH using single motor units will also have to explain five separate neuronal networks using a diagram similar to Figure 3. Adapted with permission from The American Physiological Society from Yavuz ŞU, et al (23). Copyright © 2014 The American Physiological Society. All permission requests for this image should be made to the copyright holder.

However, the PSF work also is not a perfect method for assessing the PSPs in all cases. To obtain the best estimate of the PSP profile, the discharge rate of SMU-APs should be regular and fast (please see (3) for further details about the limitation of PSF usage).

RECONSIDERING FUNCTIONAL CONNECTIONS OF THE HUMAN NEURONAL NETWORKS USING THE PSF METHOD

With the PSF method used for investigating functional connections of neuronal networks in human subjects now well recognized (41–45), several studies have been conducted to reconsider the classical literature on several issues:

1. Muscle spindle networks (the neural pathways that connect muscle spindle receptors to motoneurons) that are activated using mechanical means:
   a. Tap stimulus: using the classical methods, it has been claimed that a tap to a muscle-tendon activates a short-latency excitatory network (tendon reflex), which has a short loop that monosynaptically connects muscle spindle fibers to motoneurons. Besides, these classical experiments reported that tendon tap also activates an inhibitory network (SP) immediately after the short-latency excitatory event. The tendon reflex's amplitude and the SP's duration have been used to assess exercise-related procedures' success (see the section on "Importance of Neuronal Network Studies In Exercise Sciences"). Because we now know that the classical systems may not correctly indicate the existence, sign, and the loop length (*i.e.*, functional connections) of neuronal networks, PSF analysis must reconsider the earlier claims on movement-related neuronal networks' functional connection. Using the PSF method, it has been shown that a tap to a muscle-tendon generates only a single long-lasting excitation (excitatory network) and the so-claimed SP did not indicate the existence of an inhibitory network; rather, the SP seemed to be a

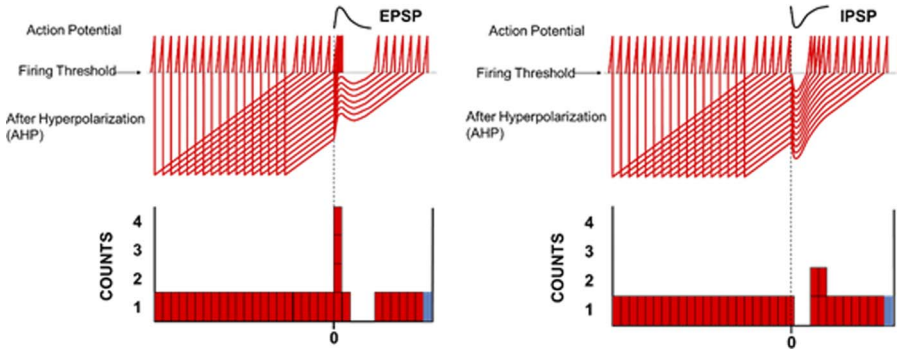

Figure 5. Noiseless motoneuron model to illustrate the errors in the classical probabilistic analysis methods. Left model: EPSP effect on regularly discharging SMU-AP. EPSP delivered at time zero adds on the depolarization trajectory and brings the SMU-APs forward and generates a peak in count numbers (classically accepted to indicate "excitation"). This forward movement of spikes effectively generates a "hole" immediately after the peak (classically accepted to indicate "inhibition"). This peak and hole then repeat a few more times due to autocorrelation function of the motor unit's discharge, generating further "excitations" and "inhibitions" (see also PSTH record in Fig. 6). Right model: IPSP effect on regularly discharging SMU-AP. IPSP delivered at time zero subtracts from the depolarization trajectory and phase delays the SMU-APs in time and generates a hole in count numbers (inhibition). This phase-delayed spikes effectively generates a "peak" immediately after the hole (excitation). This hole and peak then repeat a few more times due to motor units' autocorrelation function, generating further excitations and inhibitions (not shown).

continuation of the excitatory network and most likely involved several interneurons at various levels of the spinal cord (33).

b. Stretch stimulus: stretching a muscle briefly also activates the stretch receptors within muscles. Using the classical methods, it can be claimed that a stretch stimulus activates three excitatory (M1, M2, and M3) and two inhibitory pathways (SP1 and SP2), each of which can be asserted to have unique neuronal pathway (refer to Figs. 2–4, 7). The M1 pathway is considered to originate from the activation of thick myelinated Ia muscle spindle fibers, which synapse directly onto homonymous motoneurons (46,47). The origin of M2 in hand muscles has been claimed to be cortical (48), whereas the M2 response in lower limb muscles occurs too rapidly to be cortical (46,49). Tendon organ fibers (Ib) or group II networks may be responsible for the M2 response (50,51). The origin of the M3 response has been claimed to be the muscle spindle network that traverses the motor cortex (52). Although reports are questioning the authenticity of the first SP, which follows the M1 response (53,54), an SP after M2 response, despite expectations, has never been documented. The reasons for not recognizing the SPs in some of the earlier work maybe because most previous stretch reflex studies involved relaxed muscles with a low level of EMG, which makes observing inhibitory responses difficult (49,55). For its part, a PSF analysis shows that the stretch stimulus only activates two pathways, both of which are excitatory (Fig. 8). Once again, the realization that only two pathways are activated when a muscle is stretched should be examined further so that the correct network activated when a muscle is stretched be identified and used in exercise science investigations.

2. Muscle spindle networks activated by electrical means: investigations using classical methods concluded that, when a mixed nerve is stimulated, a short latency excitatory network (H-reflex) is activated, illustrating the connection loop of muscle spindle fibers via mono-/oligosynaptically to motoneurons (56–59). After that network's activation, the stimulus was believed to trigger a long-duration inhibitory network, the so-called SP, which is likely to contain several interneurons (56,57). On the other hand, the PSF method illustrated only a single long-lasting excitatory pathway

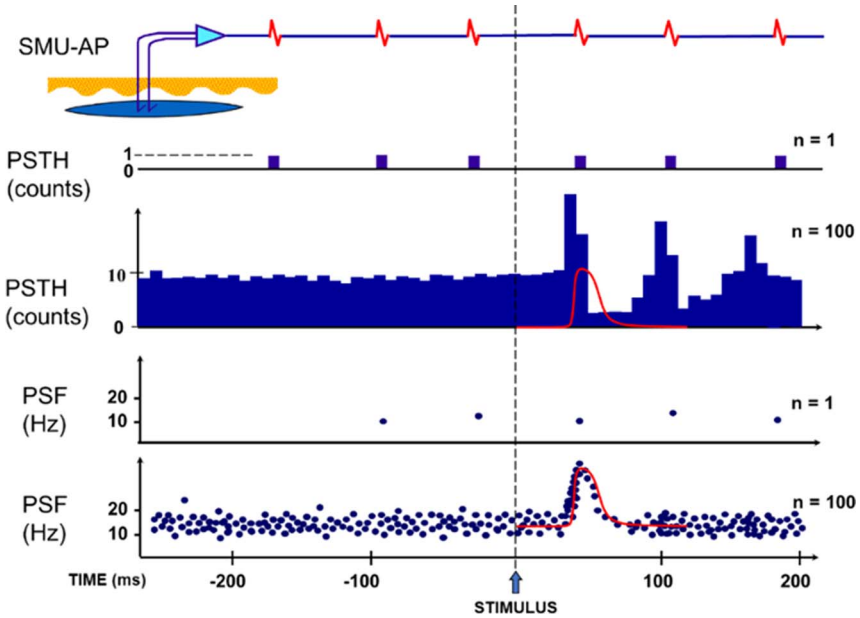

Figure 6. Building of PSTH and PSF: topline schematizes SMU-APs recorded using intramuscular fine-wire electrodes. The second line shows the building of PSTH. PSTH is built by converting each SMU-AP into acceptance pulses (n = 1). The middle graph is made up of the addition of 100 individual PSTH records (n = 100). Forth trace illustrates the SMU-AP interspike interval (ISI) conversion into discharge rates (rate = 1/ISI). Note that the discharge rate starts from the second SMU-AP and placed on the location of the second SMU-AP. The bottom trace is made up of the superimposition of 100 individual PSF graphs (n = 100).

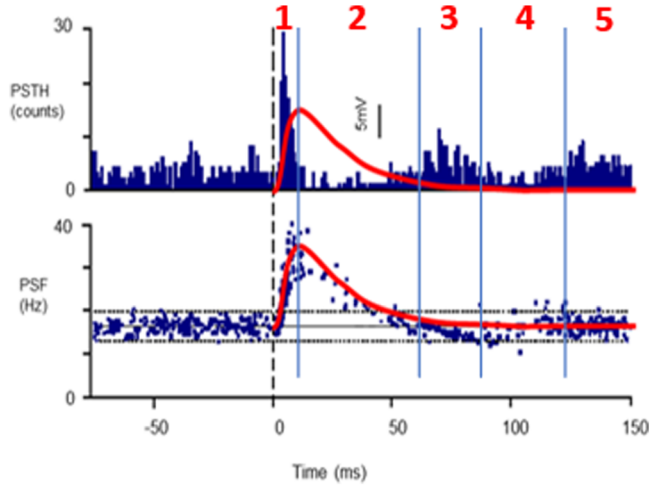

Figure 7. Direct comparison of the two methods used to study neuronal networks in human subjects indirectly. To determine which of the analysis methods gives the genuine shape of networks, known potentials (shown in grey here) were inserted into motoneurons in rat brain slices. Action potential output from the motoneuron is analyzed using both PSTH and PSF methods. Although a simple EPSP was inserted into the neuron in the case shown in the figure, the PSTH analysis indicated five separate significant peaks and troughs (1–5). On the other hand, the PSF approach indicated only a single long-lasting excitatory event, which correctly illustrated the input potential. Therefore, it is considered that PSF should be used rather than PSTH or SEMG, as it represents the synaptic events and, hence, the functional connection of neuronal networks more correctly. Adapted with permission from Elsevier from Türker KS, et al (38). Copyright © 2005 Elsevier. All permission requests for this image should be made to the copyright holder.

(H-reflex) and identified that the SP was a part of the excitatory network activated by the electrical stimulation (58,59) (Fig. 9). As outlined in the section on "Importance of Neuronal Network Studies in Exercise Sciences," several claims have been made concerning exercise training effects based on the H-reflex strength or the SP's duration. These claims will have to be reconsidered using the PSF method.

3. Networks of pain receptor origin: when pain receptors are activated using electrical or laser stimuli, the SEMG of neighboring muscles displays an intricate response pattern. Research using classical methods claimed that these stimuli activated an inhibitory network, cutaneous SP, followed by a display of an excitatory network (rebound activity). Using the same data set, a PSF analysis indicated that pain fiber stimulation activates only a single long-lasting inhibitory network (60,61). Previous studies using classical methods have indicated the importance of cutaneous reflexes in exercise training (17,18). However, in the light of the PSF findings, such experiments will have to be repeated to establish the normal ranges of the cutaneous reflexes and whether they can be used in exercise-related studies.
4. Renshaw circuitry: PSF analysis has shown that this circuitry's duration is much longer than previously thought and may involve supraspinal pathways (62). The loop length calculations of the network cannot be performed using the classical methods as the delayed spikes can peak during the late phases of the IPSP. Accurate measurements of the neuronal networks' lengths are vital as they indicate the extension of the neuronal networks' loops. Using the PSF method, we have recently discovered that the Renshaw circuitries of patients with amyotrophic lateral sclerosis are considerably shortened, and this phenomenon may be used as a diagnostic tool (63). Studies on the strength and length of the Renshaw circuitry in sports sciences have yet to be undertaken. Because this circuit represents a spinal inhibitory network of significance, any alteration in its function would affect motoneuron performance, hence indirectly causing muscle strength/resilience problems.
5. Networks that connect motor cortex to the spinal motoneurons: TMS has been used to study these networks. Studies using classical analysis methods have claimed that TMS excites a short-latency excitatory pathway, referred to as the MEP, followed by a long-lasting inhibitory network, the so-called CSP. Size of the MEP and the duration of the CSP have been used in many sports science areas to judge the modulation in the level, quality, fatiguability, and strength of muscles due to exercise training (see section on "Importance of Neuronal Network Studies in Exercise Sciences," for the details). However, using a PSF analysis, we have demonstrated that the TMS activates only a long-lasting net

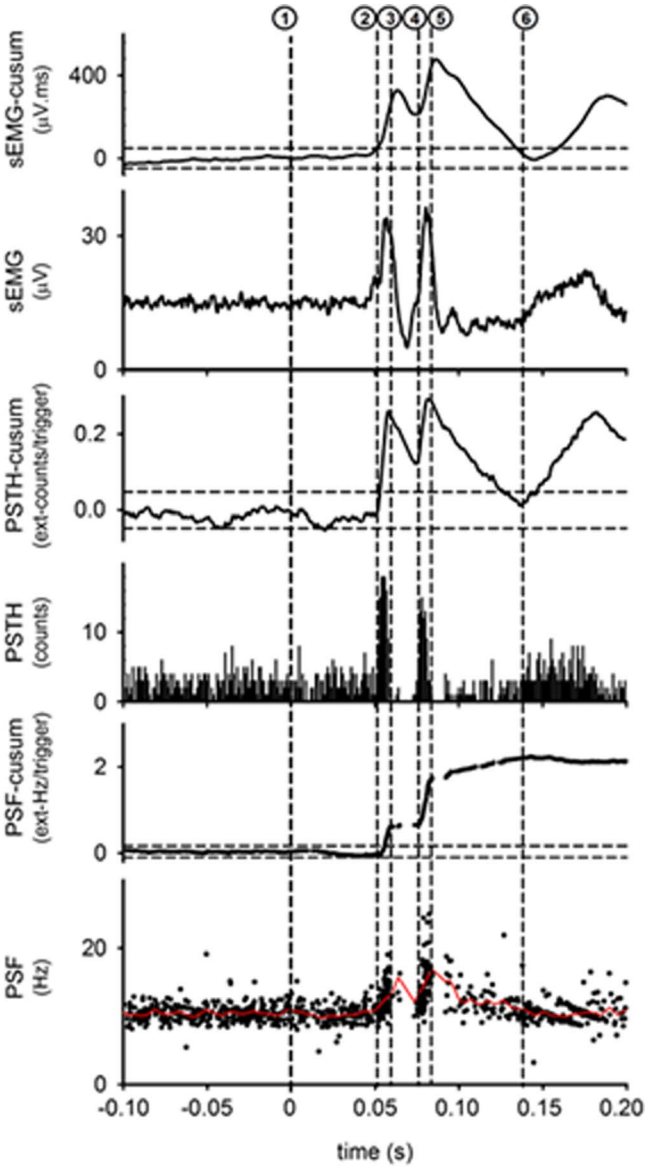

Figure 8. Probability- and discharge rate–based analyses are shown for comparison. Tibialis anterior muscle response to a stretch stimulus. Extension of Figure 2. Although the SEMG and PSTH and their CUSUMs show five significant events, indicating five separate neuronal pathways, the PSF and its CUSUM indicate only two separate significant events, both excitatory (depicted by vertical dashed lines (2–3 and 4–5)). Because the PSF relies upon significant changes in a motor unit's discharge rate due to the stimulus, only two neuronal pathways can be claimed in instances where the PSF CUSUM was the only analysis. Because neuronal network studies in human subjects need to be performed indirectly, the method must be reliable. Adapted with permission from The American Physiological Society from Yavuz ŞU, et al (23). Copyright © 2014 The American Physiological Society. All permission requests for this image should be made to the copyright holder.

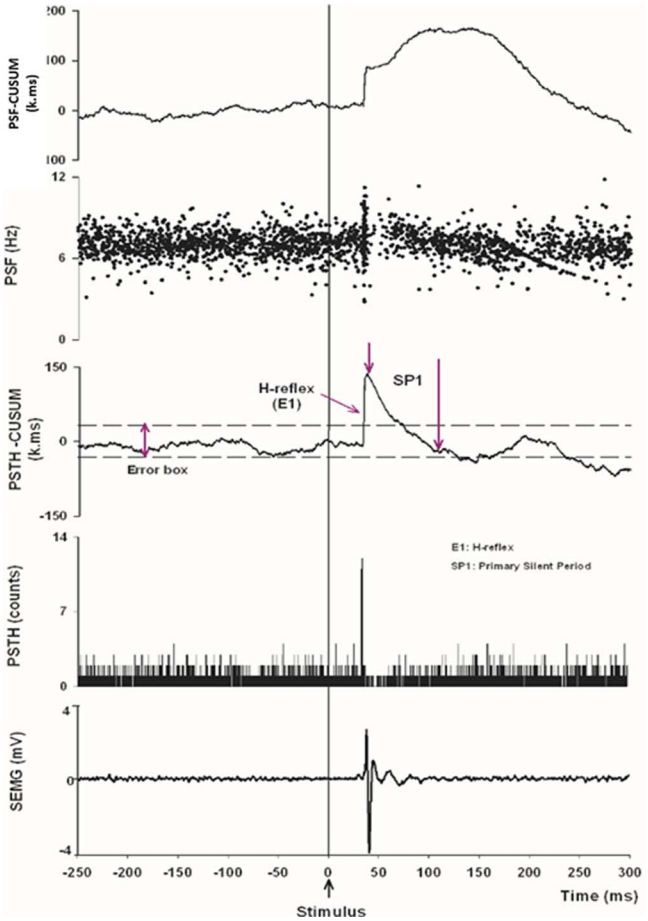

Figure 9. H-reflex result from a volunteer. From bottom to the top: SEMG in which only the H-reflex can be observed; PSTH and its cumulative sum (PSTH-CUSUM), which indicate an increase in number of spikes during the H-reflex and a reduction immediately after the H-reflex, the SP; PSF and its cumulative sum (PSF-CUSUM), which show the existence of increased discharge rate during the H-reflex and during the SP, which disclose a long-lasting net excitatory effect. Adapted with permission from Springer Nature from Binboğa E, et al (59). Copyright © 2011 Springer Nature. All permission requests for this image should be made to the copyright holder.

excitation followed by a delayed net inhibition (64,65) (Fig. 10). Here, we include a figure from one of our publications to illustrate the difference between the two methods of analyses. The same data set was used by both methods; classical methods confirmed the classical findings, that is, MEP + CSP. In contrast, the PSF method showed a long-lasting excitation followed by a delayed inhibition. Therefore, it is recommended that exercise science workers need to use the PSF method to reconsider the importance of exercise on brain networks.

We need to warn the readers regarding the limitations of our PSF-based TMS study. In our TMS study, we could only use stimulus intensities of up to about 40% of the stimulator's maximal output (around 150% of the active motor threshold). We found that more potent TMS stimuli induced a response not only on the regularly discharging SMU-APs but also on other previously nonactive single motor units. Therefore, high-level TMS pulses generated a field potential at the MEP latency, making recognition of SMU-APs impossible. This limitation restricted us from directly comparing the MEP size and CSP duration with the literature because most published works used much stronger stimulus intensities than our TMS studies. However, we are confident that threshold and low-level suprathreshold TMS stimulation induces a long-lasting net excitation followed by a period of net inhibition (65). We hope that with the new scanning SEMG techniques, it may be possible to examine the effect of high-level TMS stimuli on SMU response using the PSF method.

6. Networks connect tendon organs to the spinal motoneurons: Activity in tendon organ afferents has been known to inhibit the homonymous motoneurons. Unlike the claim made by studies

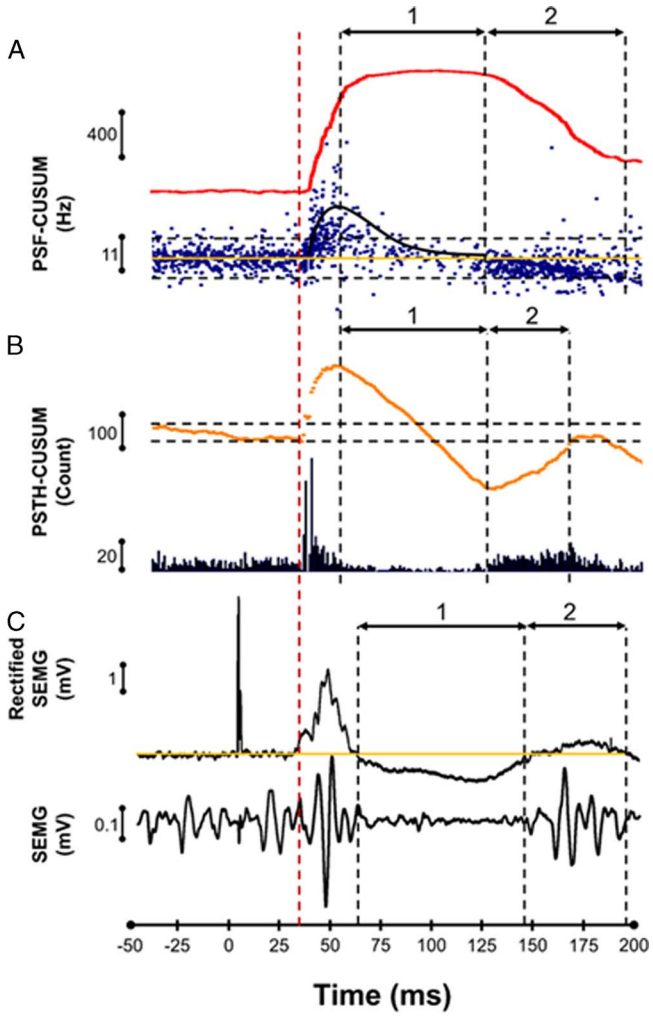

Figure 10. Representation of MEP and CSP. Black vertical dashed lines indicate the post-MEP event onsets and end points, whereas the first (grey) dashed line indicates MEP latency. Black horizontal arrows present CSP (1) and rebound activity period (2). A. PSF illustrates the frequency pattern of the unit, together with its CUSUM (top trace). The unit's discharge rate is higher than the prestimulus discharge rate during CSP (definition of net excitation). As discussed in our TMS article (65), this net excitation period may be caused by the activation of several EPSPs and IPSPs. During the rebound activity period, the unit's discharge rate falls below the prestimulus discharge rate (definition of net inhibition). As discussed in our TMS article (65), this net inhibition may be caused by the activation of several IPSPs and EPSPs. Horizontal dashed lines indicate 2 × SD according to the prestimulus firing rates, and the grey line is average background discharge rate, which was 9.8 Hz. B. PSTH represents the firing probability of the unit, represented with its CUSUM (grey Panel B). CSP is visible in this record and hence claims that low level of activity represents activation of an inhibitory pathway within the motor cortex during CSP. Dashed lines in CUSUM indicate the error box limits (12). C. Averaged-rectified SEMG and its CUSUM response show the MEP latency and CSP. Reprinted with permission from Özyurt MG, et al (65). Creative Commons Attribution 4.0 International Public License.

using classical methods, that is, a short loop inhibitory network that is activated when tendon organs in muscles are activated, PSF data suggest that tendon electrical stimulation triggers a much longer-lasting inhibition, most likely through the autogenic inhibitory reflex pathway mediated by a group I tendon afferents (66,67). Because tendon organs can be described as the "brakes" of muscle contraction, these organs' genuine pathways have the potential to be of interest for sports science researchers in determining the workings of the brake system in muscles and their importance in strength/resilience activities.

FUTURE PERSPECTIVES COMBINING THE PSF METHOD WITH NONINVASIVE SEMG-BASED MOTOR UNIT RECORDING TECHNIQUES

As detailed in the section on "Reconsidering Functional Connections of the Human Neuronal Networks Using the PSF Method," several studies have already used the PSF method to revise movement-related neuronal networks' functional connection in the human nervous system. Besides my laboratory in Istanbul, five other motor control laboratories worldwide have started to use this technique to revise some "established" neuronal networks (41–45). Only after establishing the correct range of values for a neuronal network using PSF analysis can one quantify any change in them due to an exercise test. It is hoped that more laboratories will take up this technique so that the functional connections of neuronal networks will be established and used with confidence.

Until recently, invasiveness was the major problem that prevented wide use of the PSF method. Intramuscular fine-wire electrodes were used to obtain SMU-APs during the PSF method development and its application until recently. New technologies have been developed in the last decade to obtain SMU-APs using noninvasive SEMG methods (68,69). These methods demonstrate that at least at low muscle contraction levels, it is possible to record SMU-APs using SEMG electrodes.

More recently, researchers have started to use the PSF method on reflexes using motor units obtained from SEMG techniques (70,71). In one of these articles, it has been shown that the SMU-APs obtained using the SEMG method could be used successfully in examining reflex pathways. These articles also compared and contrasted PSF results of single motor units obtained using the surface and intramuscular methods simultaneously. The outcome was remarkably similar (70). Hence, now more experiments are on their way to obtain functional connection of more extensive neuronal networks that synapse on a large portion of the motor pool innervating a muscle.

These studies will also open up a path for sports scientists to use the surface SMU techniques and the PSF analysis method to obtain adequate details of the neuronal networks that connect peripheral receptors and the motor cortex to various motoneuron pools. Based on this knowledge, they can then discover movement strategies to modulate these networks to the benefit of athletes.

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