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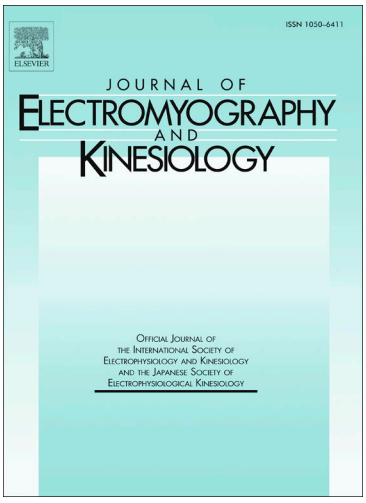

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PII: S1050-6411(20)30102-4
DOI: https://doi.org/10.1016/j.jelekin.2020.102460
Reference: JJEK 102460

To appear in: *Journal of Electromyography and Kinesiology*

Received Date: 16 May 2020
Revised Date: 5 August 2020
Accepted Date: 19 August 2020

Please cite this article as: M.G. Özyurt, B. Topkara, B.S. Şenocak, A.S. Budan, M.N. Yüce, K.S. Türker, Post-activation depression of primary afferents reevaluated in humans, *Journal of Electromyography and Kinesiology* (2020), doi: https://doi.org/10.1016/j.jelekin.2020.102460

Post-activation depression of primary afferents reevaluated in humans

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Running title: H-reflex Depression in Humans

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Abstract

Amplitude variation of Hoffmann Reflex (H-reflex) was used as a tool to investigate many neuronal networks. However, H-reflex itself is a subject to intrinsic changes including post-activation depression (P-AD). We aimed to investigate P-AD and its implication on motor control in humans. Upon tibial nerve stimulation in 23 healthy participants, peak-to-peak amplitude change of H-reflex was investigated using surface electromyography (SEMG) of soleus muscle. Variety of stimulus intensities, interstimulus intervals (ISIs), voluntary contraction levels/types and force recording were used to investigate the nature of P-AD. We have shown that P-AD was significantly stronger in the shorter ISIs. The only exception was the ISI of 200 msecs which had a weaker P-AD than some of the longer ISIs. Sudden muscle relaxation, on the other hand, further increased the effectiveness of the ongoing P-AD. Moreover, P-AD displayed its full effect with the first stimulus when there was no muscle contraction and was efficient to reduce the muscle force output by about 30%. These findings provide insight about the variations and mechanism of P-AD and could lead to improvements in diagnostic tools in neurological diseases.

Keywords: electrical stimulation; electromyogram; H-reflex; motor control; soleus muscle

Introduction

Hoffmann Reflex (H-reflex), electrical analog of the stretch reflex, is elicited by low-intensity electrical stimulation of the thickest myelinated nerve fibers (i.e. Ia fibers) and bypasses muscle spindle receptors to induce Ia excitatory postsynaptic potential (Ia-EPSP) in homonymous motoneurons. This reflex is one of the best-characterized and most frequently-used tools for studying neuronal networks (Hoffmann 1910; Hoffmann 1918). Although H-reflex has been studied widely, many physiological parameters should be considered when eliciting H-reflex, including presynaptic modulation of the Ia-EPSP (Hultborn et al, 1996; Iles 1996).

One of the most frequently studied presynaptic mechanisms is the classical GABAergic inhibition that reduces the effectiveness of spindle primary endings on motoneurons (Eccles et al, 1963; Rudomin and Schmidt 1999). This presynaptic inhibition (PSI) occurring at Ia afferent terminals has a duration of a few hundred milliseconds (Eccles 1964; Hultborn et al, 1996; Morin et al, 1984). The other type of presynaptic inhibition mechanism with a relatively long duration is known as post-activation depression (P-AD) (Crone and Nielsen 1989) that can last as long as 8 seconds (Burke et al, 1989; Crone and Olesen 1982; Hultborn et al, 1996). Various means of Ia fiber activation such as electrical stimulation with short interstimulus intervals (ISIs) (Magladery et al, 1952), passive lengthening of the muscle (Schieppati and Crenna 1984), tendon taps (Katz et al, 1977) or transcutaneous spinal stimulations (Andrews et al, 2015) can induce P-AD of the primary afferents.

Both PSI and P-AD are attenuated in individuals with neurological disorders displaying spastic symptoms such as by stroke and spinal cord injury as well as amyotrophic lateral

sclerosis. Hemiplegic patients, for instance, have been found to exhibit attenuated PSI and P-AD (Aymard et al, 2000; Tahayori et al, 2015). In addition, these mechanisms are less pronounced in multiple sclerosis, spinal cord injury (Grey et al, 2008; Morita et al, 2001; Nielsen et al, 1995) as well as in amyotrophic lateral sclerosis (Hedegaard et al, 2015; Iles and Roberts 1986). These findings indicate that the mechanism underlying PSI and P-AD are affected by some neurological diseases, and therefore, they can be used in clinics to assess disease progression.

Although there is a consensus about the mechanism of PSI, i.e., presynaptic axo-axonal synapse on GABA receptors that inhibit neurotransmitter release (Dudél 1963; Kretz et al, 1986; Parnas et al, 2000; Romanò and Schieppati 1987; Schieppati and Crenna 1984), several explanations have been put forward to explain P-AD. It has been suggested that it might be caused by, but not limited to, neurotransmitter depletion at Ia terminals (Katz et al, 1977; Mark et al, 1968) or by recurrent inhibitory systems (Ishikawa et al, 1966). Although there is no general agreement regarding the mechanism underlying P-AD, it has been concluded that P-AD is not a postsynaptic phenomenon but that it occurs at the presynaptic level (Hultborn et al, 1996; Schieppati and Crenna 1984).

There are still gaps to be filled to understand P-AD and its full implications in health and disease, including its detailed temporal characteristics, various cortical influences on it, and its effect on force generation. In this study, we used soleus H-reflex to evoke P-AD to understand its control on primary afferent output to motoneurons. We hypothesize that the nature of the voluntary contraction, e.g. contraction level and contraction evoked before or during P-AD has been activated, may affect the level of P-AD. We also hypothesize that P-AD is effective to reduce muscle force generation as it reduces the

primary afferent input on motoneurons. Lastly, we hypothesize that the relative strength of P-AD is dependent on stimulus intensity as well as has inverse relationship with ISI.

Methods

Recording and setup

CED 1902 Quad System MKIII amplifier and CED 3601 Power 1401 MKII DAC were used for recording. Spike2 7.20 software was used for data acquisition and analysis (Cambridge Electronic Design, England). A constant current stimulator (model DS7A, Digitimer Ltd, Hertfordshire, UK) was used for electrical stimulation. Isometric force during dorsiflexion of the right foot was measured using a linear strain gauge (Model LC1205-K020, A & D Co. Ltd., Tokyo, Japan: linear to 196 N).

In order to record the activity of the soleus muscle, surface electromyography (SEMG), which was sampled at 20,000 Hz and filtered online with 20-10,000 Hz band-pass filter, was used. After skin preparation, which consisted of rubbing the skin with sandpaper, cleaning the surface with alcohol and applying conductance gel to decrease impedance, two standard SEMG electrodes (Ag/AgCl) were placed on the soleus muscle.

In addition to SEMG, we recorded the twitch force generation for the twitch protocol from the force transducer located under the foot. Force signals were amplified (x 1,000), filtered (DC-100 Hz), and sampled at 2,000 Hz using the same data acquisition system. Moreover, during the tibial nerve stimulation, we recorded intramuscular EMG response from tibialis anterior (TA) muscle to investigate if the tibial nerve stimulation evokes a response in antagonist TA. Using similar preparation procedure, we placed two SEMG electrodes on TA muscle with 4 cm interelectrode distance (Tucker and Türker 2005).

Between SEMG electrodes, a pair of fish-hooked fine wire insulated electrodes (5 mm tip peeled, 75 μm in core diameter; Medwire, USA) was inserted using 25-G surgical needle after sterilized with autoclave. Intramuscular EMG signals of TA were sampled at 20,000 Hz and filtered online with 200-10,000 Hz band-pass filter.

Experimental design

The Human Ethics Committee at Koç University approved the study procedure in accordance with the Declaration of Helsinki. All experiments were performed in the neurophysiology laboratory located at the Koç University Medical School on subjects who signed informed consent forms. All subjects were in the 18-35 age groups and the experiment was performed on the right leg of subjects. Some of the subjects participated in more than one experimental protocol. Exclusion criteria were; use of medication for any type of neurological problem, known neuromuscular disorder and chronic leg or back pain. The protocols were followed randomly.

Common protocol

Subjects rested comfortably in the prone position on an examination bed and were asked to relax or contract their soleus muscle during the experiment by performing plantar flexion against a stabilized panel (force transducer) at the foot of the bed. After measuring the horizontal distance between the condyles, a cathode (3×3 mm) was placed at the midpoint of an imaginary line across the popliteal fossa at the crease level and an anode (10×12 cm) was placed immediately proximal to the patella to evoke the H-reflex by monopolar stimulation of the tibial nerve (Özyurt et al, 2018). Electrical stimuli were delivered as square pulses with a width of 1 msec. Hmax and Mmax were obtained by increasing the stimulus intensity during the relaxed state.

Subjects were asked to perform 3 maximum voluntary contractions (MVCs) each lasted for 3 secs and were separated by a rest period of 1-minute. During the MVCs, subjects were asked to perform maximal plantar flexion against stabilized panel at the foot of the bed as strongly as they could. Then, 3-sec EMG recordings were rectified and smoothed with a time constant of 0.1 secs, and the maximal peak-to-peak value was taken as 100% MVC. This smoothed-SEMG recording was provided to subjects as real-time visual feedback whenever voluntary contractions were needed during the protocol. Individual H-reflex responses were quantified by measuring the peak-to-peak amplitudes of non-rectified SEMG recordings. Peak-to-peak amplitudes were normalized according to the amplitude of the first H-reflex obtained in each set. Normalized values for all subjects in each set were averaged for each protocol. Shapiro-Wilk test was used to investigate the distribution of the data. The level of significance was selected as $p < 0.05$. All statistical analyses were two-tailed and performed using GraphPad Prism 8 software on the normalized and averaged data.

Interstimulus interval protocol

Ten subjects (4 female and 6 male) participated in this experiment. The effect of P-AD on the amplitude of the H-reflex was investigated using 9 different ISIs without voluntary contraction. The ISIs were chosen to be 25, 50, 100, 200, 500 msecs and 1, 2, 5, 10 secs. We delivered a paired stimulus at one of these ISIs, using a stimulus intensity of Hmax/2. For each of the ISI, the experiment was repeated 3 times per subject, with a rest period of 10 secs between each repeat, for each subject. In total, 30 H-reflex measurements from 10 subjects were obtained per ISI. The second H-reflex amplitude obtained with each of these ISIs was compared with the second H-reflex amplitude obtained with the ISI of 10 secs, which was defined as the P-AD-free control. Repeated-measures (RM) one-way

ANOVA with Dunnett's multiple comparisons test was used to investigate the changes in H-reflex amplitudes of each ISI compared to H-reflex amplitudes obtained at 10 secs ISI (as the reference ISI) based on 10 subjects. Also, paired t-test was performed to assess the differences between ISIs of 200 and 500 msecs.

Stimulus intensity protocol

Effect of stimulus intensity on P-AD during the relaxed state was investigated using 3 different intensities, i.e. Hmax, Hmax/2 and Hmax/5, in 11 subjects (5 female, 6 male) for ISI of 1 sec with 10 secs rest time in between each of the pairs. Similarly, H-reflex responses obtained in first stimulus of each pair were compared in order to determine the variability of the H-reflex during the experiment. For statistical analysis RM one-way ANOVA with Tukey's multiple comparisons test was used.

Long period of stimulation protocol

For ISIs of 1 and 10 secs, we evoked the H-reflex continuously for 50 secs to investigate timewise change of P-AD in 8 subjects (2 female, 6 male). This protocol was performed in the relaxed muscle state and 30% of the subject's own MVC. Between each 50 secs protocol, subjects rested for 2 minutes. Due to long stimulation times, each ISI and MVC level was tested only once for each subject. For statistics, RM one-way ANOVA with Dunnett's multiple comparisons test was used to test timewise reduction in H-reflex due P-AD for each combination of parameters.

Protocol for contraction followed by instant release during stimulation

Seven subjects (3 female, 4 male) were asked to perform voluntary contractions at the level of 5, 30 or 50% MVC during stimulation (Hmax/2 intensity, ISI of 1 sec). After

delivering 5 stimuli in the relaxed state, subjects were asked to contract her/his muscle for the duration of 5 stimuli at one of the above-mentioned contraction levels. This was followed by another 4 stimuli delivered during the relaxed state. The first stimulus in the pre-contraction relaxed state was used to detect the control/unaffected H-reflex level and not included in the comparison analyses. In sum, there were 4 pre-contraction, 5 contraction and 4 post-contraction H-reflexes produced per subject's trial. Each subject repeated each contraction protocol 5 times. The average of pre-contraction, contraction and post-contraction reflex amplitudes were compared using Friedman test. In addition, average of 4 pre-contraction levels was compared with immediate release level (instant release) using paired t-test.

Twitch protocol

To measure the amount of twitch force generated by muscle contraction, we delivered paired electrical stimuli with an ISI of 1 sec (Hmax/2 intensity) to the tibial nerve at rest while recording SEMG from soleus and intramuscular EMG from TA, the latter for detecting the possible crosstalk. We applied 5 paired stimuli with 1 sec of ISI within each pair but 10 secs between each pair for each of the six subjects (2 female, 4 male). The first and second twitches were compared individually using Mann-Whitney test.

Results

Effect of ISI on P-AD

To understand the temporal characteristics of P-AD, various ISIs were applied to stimulate primary afferents (**Figure 1**). Each ISI was applied as a pair. H-reflex amplitude was found to be dependent on interstimulus interval ($p < 0.0001$, RM one-way ANOVA).

As 10 secs of ISI has been suggested to be P-AD-free (Hultborn et al, 1996), we used this ISI as the reference H-reflex value and the values obtained using other ISIs were compared with this reference value. A significant reduction in H-reflex amplitude was found in ISIs from 5 secs to 25 msecs (10 secs vs 5 secs: $p = 0.0156$; 10 secs vs 200 msecs: $p = 0.0036$, 10 secs vs the rest: $p < 0.0001$). ISIs of 50 and 25 msecs did not evoke any observable H-reflex in response to the second stimulus (**Figures 1A and 1B**).

A dramatic recovery of H-reflex amplitude in 26 out of 30 trials from 10 subjects was detected in ISI of 200 msecs. H-reflex was significantly less affected by P-AD when the ISI of 200 msecs was used compared to a longer ISI of 500 msecs ($p < 0.0001$) **(Figure 1C)**.

P-AD and primary afferent stimulation intensity was inversely proportional

P-AD-based reduction in H-reflex was investigated using various stimulus intensities (**Figure 2**). To test the effect of stimulus intensity, an ISI of 1 sec and 10 secs were used to evoke two consecutive H-reflexes for P-AD. For the ISI of 1 sec, the least-affected H-reflex obtained with Hmax intensity which evoked an amplitude of 58.6 ± 17.2% (mean ± SD) of the first H-reflex (H_{first}) which was significantly higher than Hmax/2 (31.9 ± 18.7%) and Hmax/5 (25.2 ± 17.6%) ($p < 0.0001$). On the other hand, an ISI of 10 secs showed no detectable reduction when different stimulus intensities were used ($p > 0.1$ for all intensities) (**Figure 2B**).

Maximum effect of P-AD was immediate, and its effect was dependent on the ISI and voluntary contraction for long period stimulation

H-reflex reduction due to P-AD at different contraction levels and ISIs was investigated by evaluating the amplitude of H-reflexes evoked consecutively, for a duration of 50 secs.

P-AD was almost absent in sets with an ISI of 10 secs and/or during voluntary contraction at 30% of MVC. We noted that after the second reflex response, there was no further decrease in the amplitude of H-reflex in most sets **(Figure 3)**.

MVC level of 0% MVC: The ISI of 1 sec resulted in a large decrease in the amplitude of the H-reflex at the relaxed state (RM one-way ANOVA, $F(2.832, 19.82) = 15.34$, $p < 0.0001$). The amplitude of the second H-reflex fell to less than half of the first H-reflex during the relaxed state where all of the consecutive H-reflex values were significantly smaller than the first H-reflex (all $p < 0.05$). On the other hand, P-AD (starting with the second H-reflex) did not significantly change with time (all $p > 0.05$) proving that the maximum P-AD is starting to take place with the first test H-reflex following control stimulus (**Figure 3A and 3B**). However, ISI of 10 secs at the relaxed state had similar H-reflexes over time (RM one-way ANOVA, $F(2.217, 15.52) = 0.3793$, $p = 0.7111$, and for all multiple comparisons $p > 0.1$).

MVC level of 30% MVC: Although 30% MVC voluntary contraction reduced the effect of P-AD on H-reflex, a slight decrease in the amplitude of following the second reflex was also seen during ISI of 1 sec but not in ISI of 10 secs (**Figure 3C and 3D**). For ISI of 1 sec, all the H-reflex responses following the first stimulus were not affected by P-AD (RM one-way ANOVA, $F(3.612, 25.29) = 0.7636$, $p = 0.5469$). ISI of 10 secs also evoked similar H-reflex amplitudes for all the time period as in relaxed state (RM one-way ANOVA, $F(2.878, 20.15) = 0.7987$, $p = 0.5044$, individual time points had all $p > 0.5$).

Voluntary contraction reversed the H-reflex reduction

We investigated the effect of sudden contraction during continuous stimulus delivery influenced P-AD **(Figure 4)**. We found that a weak contraction **(Figure 4A)** reduced P-AD significantly and the H-reflex level returned to its original state upon relaxation ($p = 0.0007$). Stronger contraction levels, such as 30% MVC **(Figure 4B)** and 50% MVC **(Figure 4C)** not only eliminated the effect of P-AD (for both MVC levels: $p < 0.0001$) but also facilitated the H-reflex such that the reflex had a greater amplitude than the control level (i.e. evoked by the first stimulus - blue dashed lines in **Figure 4**). As soon as the contraction stopped (instant release), a significant fall of the H-reflex amplitude to below the pre-contraction level was observed. This reduction was significant for all contraction levels ($p < 0.05$).

P-AD reduced force generation

To illustrate the effectiveness of the reduced reflex on force generation (**Figure 5**) we first checked the possibility of crosstalk between soleus and tibialis anterior muscles (Türker and Miles 1990). Since surface EMG electrodes on TA are susceptible to detect stimulus-evoked activities on triceps surae muscles (Türker and Miles 1990), we used peeled-tip intramuscular EMG electrodes (multi-motor unit: MMU) to detect accuracy of the tibial nerve stimulation, i.e. whether we stimulate common peroneal nerve as well. The results indicate that there was little to no activity in TA intramuscular EMG during electrical stimulation of the tibial nerve (**Figure 5A**). Pairs of stimuli with 1 sec of ISI (Hmax/2 intensity) were delivered to the tibial nerve to evoke H-reflex and force generation under influence of P-AD (**Figure 5B**). Twitches evoked by the first and the second stimuli were compared. Twitch force generated by the second stimulus

significantly reduced to about 69.7 ± 35.6% of the level of the first stimulus. Whereas the second H-reflex was found to be 31.9 ± 18.7% of the first H-reflex, reported in the previous section.

Discussion

Previous findings in the literature have revealed the importance of temporal properties of P-AD and the effect of voluntary contraction on it (Burke et al, 1989; Clair et al, 2011; Crone and Nielsen 1989). We have confirmed the findings of others on P-AD that it is most pronounced in the relaxed muscles and is less effective during voluntary contraction. Our study elaborated these earlier findings and discovered several new findings. Firstly, along with our first hypothesis, there was a clear recovery at the H-reflex if the primary afferents were stimulated with an ISI of 200 msecs during voluntary contraction, and sudden relaxation increased the effect of ongoing P-AD. Secondly, along with our second hypothesis, we have discovered that P-AD operates to reduce the contraction strength of the muscles evoked by the synchronous discharge of ipsilateral muscle spindle afferents. Thirdly, agreeing with our third hypothesis, we have established that P-AD is more pronounced when lower number of primary afferents are activated, inversely related to ISI except for the ISI of 200 msecs, and shows its maximal effect immediately following the first stimulus.

The case for P-AD

Previous studies propose that attenuation of the effectiveness of the primary afferent activity by P-AD might be due to neurotransmitter depletion (Hultborn et al, 1996; Katz et al, 1977; Mark et al, 1968). An observation against the neurotransmitter depletion theory in this study would be the unchanged P-AD during continuous stimulations. P-AD

showed its full effect at the second stimulus and stayed rather constant for as long as stimulation continued. This finding may provide evidence that neurotransmitter depletion is not the main mechanism responsible for P-AD, as we would expect a gradual decrease of the response as shown by Peshori et al, (1998). Moreover, weaker P-AD at 200 msecs ISI would be another support against the neurotransmitter depletion proposal as it should have resulted in smaller H-reflex compared to longer ISIs. However, neurotransmitter depletion may contribute to it to some extent via readily available vesicles and activation of other receptors such as serotonin receptors which might contribute to P-AD (Garcia-Ramirez et al, 2014; Kaeser and Regehr 2017).

Majority of the postsynaptic inhibitory circuits evoked by a single stimulus, including but not limited to Ia reciprocal inhibition (ceases around 25 msecs; (Capaday et al, (1990); Uysal et al, (2019)), recurrent inhibition (ceases around 50 msecs; Özyurt et al, (2019)), silent period due to cutaneous input (ceases around 80 msecs; Logigian et al, (1999)), Ib inhibition (ceases around 10 msecs; Pierrot-Deseilligny et al, (1979)), does not last more than 200 msecs. Since we have performed majority of the experiments using 1-sec ISI, we believe that these mechanisms would only have a minimal effect on the H-reflex. However, these circuitries can influence the H-reflex when there is a voluntary contraction and when ISIs less than 200 msecs is used.

Similar to the findings of Tahayori et al, (2015), we have shown a recovery at the ISI of 200 msecs. Although it is difficult the interpret the reason for this recovery, it may be due to the loss of GABAergic PSI strength at 200 msecs interval (Figure 8C of Hultborn 1996). Yet, this does not explain why 200 msecs ISI has a lower P-AD than 500 msecs ISI. This could be due to the intrinsic P-AD mechanism that may have a certain timeline

to fully operate. However, with the current results, these explanations should stay only at the hypothetical level. On the other hand, high level of P-AD at ISIs lower than 200 msecs may be due afterhyperpolarization of motoneurons, recurrent inhibition and/or other oligosynaptic pathways as these systems may reduce motoneuron activity and result in reduced H-reflex in addition to classical PSI (Chamma et al, 2012; Eccles et al, 1963; Özyurt et al, 2019; Pierrot-Deseilligny and Burke 2005; Schupp et al, 2016).

Electrical activation of the primary afferents and mechanical activation of the muscle spindles can both induce P-AD (Hultborn et al, 1996). This could be the reason why we observed extra P-AD when voluntary muscle contraction suddenly ceases (**Figure 4**). Instant cease of the muscle contraction/sudden release may lead to a stretch of the muscle (due to rapid muscle elongation) hence activating muscle spindles further. This event may lead to an extra activation of primary afferents and hence generate an increase in P-AD. In addition to P-AD, these sudden length changes may activate TA spindle afferents which may cause reciprocal inhibition to reduce soleus H-reflex further.

Possible mechanisms responsible for P-AD

Inhibitory inputs are less effective if a cell is already excited. This inverse relationship is valid for both postsynaptic mechanisms like recurrent inhibition (Özyurt et al, 2019) and PSI on Ia afferents (Hultborn et al, 1987). Therefore, we investigated if a similar inverse relationship is valid for P-AD. Excitatory activity in our experiments was tested in two different ways. Firstly, we used various stimulus intensities to activate varying numbers of spindle primary afferents. When stronger stimuli are used P-AD became less effective. Secondly, we used voluntary drive and found that high level of voluntary drive reduced and even eliminated P-AD. Alteration of P-AD during voluntary contraction, therefore,

could be due to the inhibitory input evoked via descending systems on a hypothetical presynaptic P-AD mechanism, similar to a previous report on PSI (Hultborn et al, 1987).

Another supporting evidence for hypothetical presynaptic mechanism is the alteration of PSI and P-AD in spastic patients (Morita et al, 2001; Nielsen and Hultborn 1993; Yang et al, 2015). These studies have shown that PSI and P-AD are reduced after spinal cord injury or in the affected side after stroke (Lamy et al, 2009). The mechanism of action for the reduced P-AD may be similar to the cause of spasticity. It has been suggested that the spasticity involves formation of new Ia synapses on motoneurons which are free of PSI, and therefore, results in hyperreflexia (Calancie et al, 1993; Yates et al, 2011). Such findings in spastic patients would propose the involvement of an inhibitory interneuron mechanism which may not affect newly formed Ia connections on motoneurons. Nonetheless, more experiments are needed to prove/disprove this mechanism that is indirectly supported by the current and previous findings.

Although there is some evidence to support that presynaptic inhibitory mechanism may be responsible for P-AD, none of these indirect evidences explain the lack of dorsal root potential during P-AD, in contrast to PSI (Hultborn et al, 1996). This could be due to the shunting of the action potential arriving at Ia fibers or its attenuation with different mechanisms other than primary afferent depolarization (Furman 1965; Paulus and Rothwell 2016). This inhibition may occur by a similar shunting mechanism but at the motoneuron dendrite through chloride channels (Guo and Hu 2014; Paulus and Rothwell 2016) (**Figure 6**).

Another system to be discussed for the P-AD mechanism is the bistable interneurons. It is known that motoneurons are capable of propagating spontaneous action potentials in

the absence of persistent synaptic excitation (Collins et al, 2002; D'Amico et al, 2013; Kiehn and Eken 1998). These 'plateau potentials' can also occur in certain groups of interneurons that mediate long-lasting sustained activity (Bellardita et al, 2017; Hounsgaard and Kjaerulff 1992). This plateau potentials can even be observed upon dorsal root stimulation (Hounsgaard and Kjaerulff 1992), and can last as long as 5 secs (Bennett et al, 2001). However, more studies are needed to prove/rebut bistable interneuron involvement in P-AD mechanism.

Possible physiological significance of the primary afferent inhibition mechanisms

Reflexes have circuits that are too short to be willfully controlled. This makes reflexive contractions potentially dangerous since they have the capacity to generate large/damaging forces without conscious participation. To limit its maximal output, nature needs to develop local circuits to control the output of the motoneurons, hence reduce the risk of strong/damaging reflexive contraction. To do that, nature cannot rely upon the conscious mind since such reflexive contraction can occur quickly and unexpectedly. Circuitries near the motor pool would be ideal for this control. We have shown clearly that P-AD resulted in twitch force reduction that occurred at the same time as the H-reflex reduction. This finding reveals the importance of the protection/control property of P-AD from repetitively and synchronously occurring primary afferent discharges (H-reflex) and during sudden muscle elongation which may otherwise result in damaging muscle contraction. Therefore, P-AD can be a substantial protective mechanism along with other spinal inhibitory circuitries such as reciprocal, recurrent and Ib networks.

We have also found that the effectiveness of P-AD was greatly reduced during voluntary contractions, especially at higher cortical drives, thus allowing less inhibited conscious motor control (Faist et al, 1996; Hultborn et al, 1987; Katz et al, 1988; Kernell and Hultborn 1990). If a sudden stretch of a muscle is expected, such as walking on slippery stones near a beach, dynamic and static gamma systems will be activated to stiffen muscles of the leg (Prochazka et al, 1988). Resulting co-contraction of muscles would then allow finer control and reduce the risk of large muscle stretch should a slip of the foot occurs (for similar findings in jaw unloading reflex see Miles and Madigan (1983)).

We can observe the importance of these protective mechanisms when they fail in patients with spinal cord injury. Unlike in normal individuals, in spastic patients both the stretch and H-reflexes increase dramatically during the late swing phase when the soleus muscle is minimally active. Since soleus is stretched naturally in the late swing phase of walking, it triggers clonus in the spastic patients thus affecting the success of the next step (Thompson et al, 2019). In normal individuals, a stretch stimulus induces bursts of spindle primary afferents discharge lasting for up to 100 ms (Abbruzzese et al, 1985; Dimitriou 2014; Jahnke and Struppler 1989). Such strong spindle activity however does not generate large force outputs as their synaptic input onto motoneurons is inhibited via P-AD. Normally, unexpected rapid stretches of muscles occur regularly in everyday life and thanks to these protective mechanisms, stretched muscles do not contract strongly to prevent us from moving smoothly. However, these obstructive contractions do occur in patients with spinal cord injury that indicates the importance of these protective networks. The knowledge about these networks would allow researchers to device scientifically based methods to help patients.

Limitations of the study

This study investigated P-AD of the H-reflex to understand its properties in human neuromuscular system. The fact that P-AD depends on the level of voluntary drive requires future single motor unit investigation to elucidate the distribution of P-AD on varying sizes of motoneurons.

Acknowledgements

We are grateful to the subjects who volunteered to participate in this project. Also, we acknowledge the role of the Koç University School of Medicine for funding this project. We thank the Scientific and Technological Research Council of Turkey (TÜBİTAK) for funding Mustafa Görkem Özyurt under the "2211-A National Scholarship Program for PhD Students". The authors declare that they have no conflict of interest.

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FIGURES AND LEGENDS

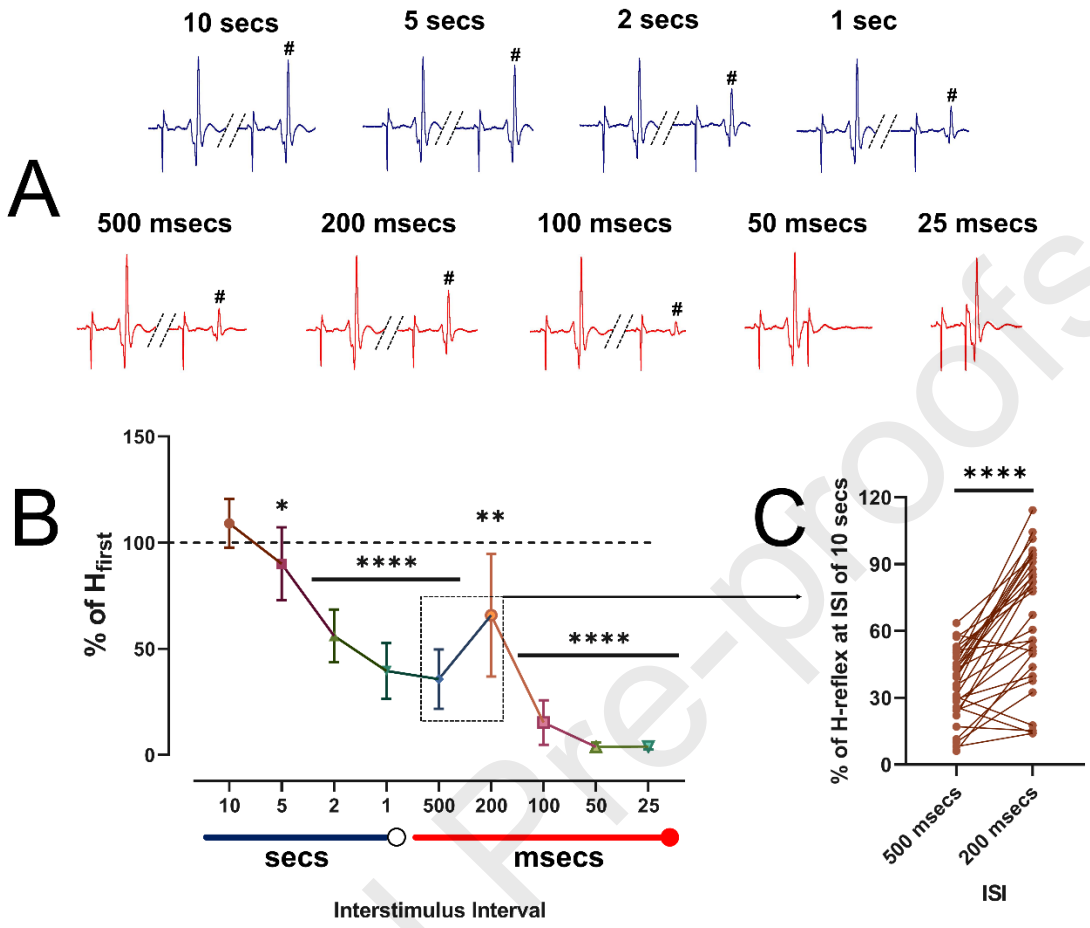

Figure 1. Changes in the H-reflex with varying ISIs*. A) Sample waveforms of the H-reflexes obtained at various ISIs. "#" indicates second H-reflex. There was no detectable H-reflex when ISIs of 50 and 25 msecs were used. B) Average values of H-reflex amplitude reduction as a percentage of H_{first} amplitude. C) Comparison of the recovered H-reflex at ISI of 200 msecs with previous ISI which is 500 msecs. P-AD of the H-reflex was observed starting with an ISI of 5 secs. Error bars show standard deviation. **** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$. N=10.*

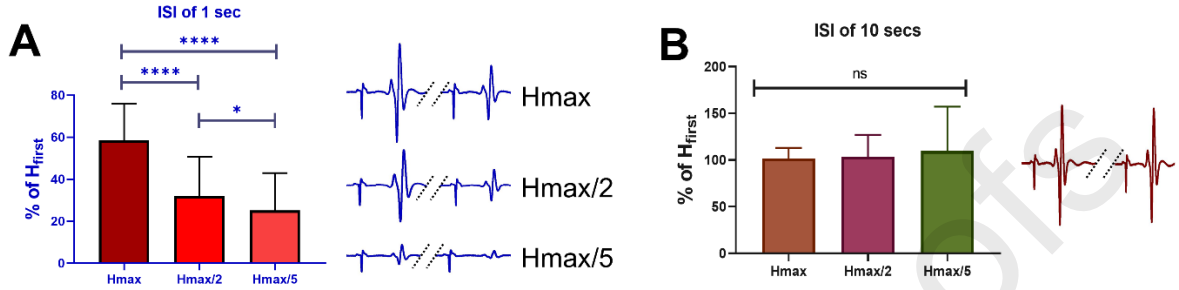

Figure 2. Change in H-reflex amplitude due to P-AD at different stimulus intensities. *The amplitude of the second H-reflex as a percentage of the amplitude of the first H-reflex when A) an ISI of 1 sec (N=11) and B) the average values of H-reflex amplitude when an ISI of 10 secs was used (N=11). Error bars are standard deviation. **** $p < 0.0001$, * $p < 0.05$, ^{ns} $p > 0.05$.*

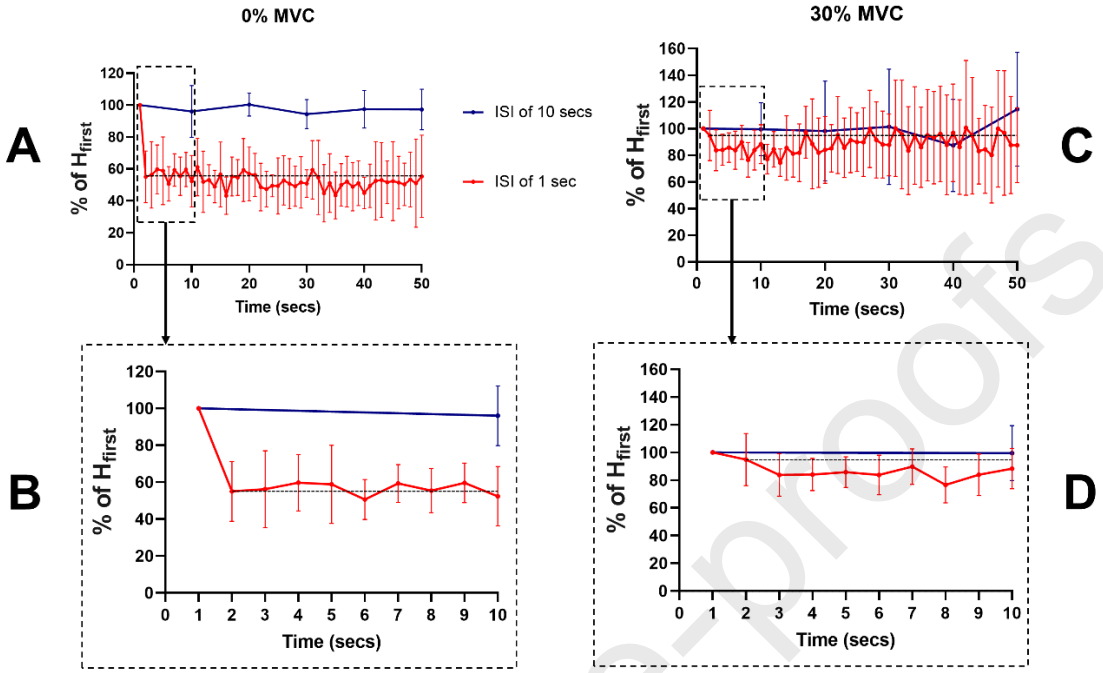

Figure 3. Successive H-reflex amplitudes elicited for 50 secs*. Each point shows average H-reflex amplitude of all subjects obtained after normalization to the first stimulus. A) 0% MVC for ISI of 1 (blue) and 10 (red) secs. B) 30% MVC for ISI of 1 (blue) and 10 (red) secs. B) Focused figure for the first 10 secs of the 0% MVC graph. D) Focused figure for the first 10 secs of the 30% MVC graph. Error bars are SD, N=8. Horizontal dashed line shows the reduced H-reflex following the control stimulus, i.e. response at second stimulus.*

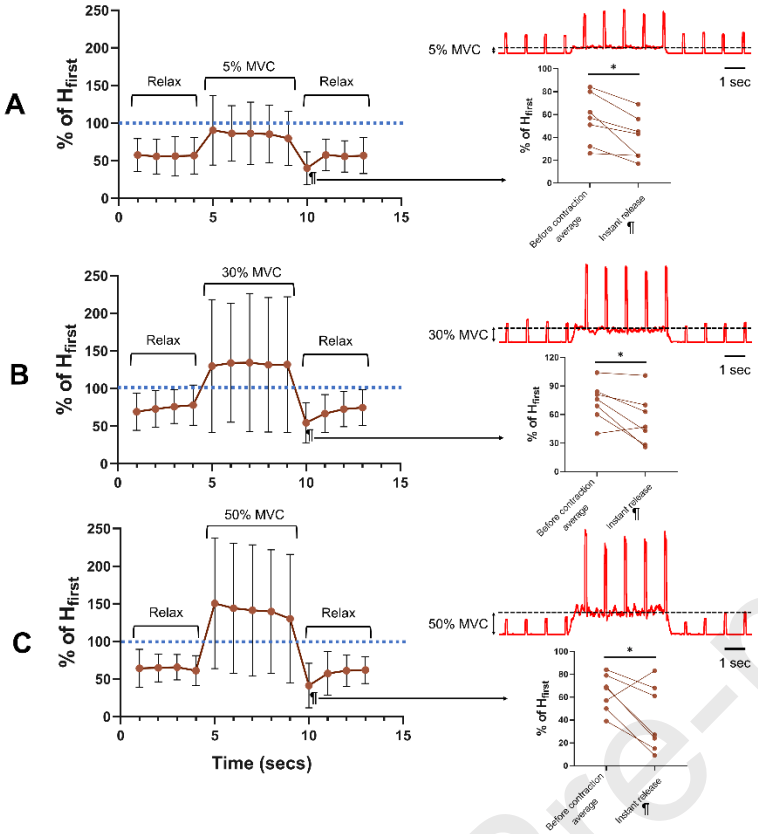

Figure 4. Effect of weak and strong contractions on H-reflex during continuous electrical stimulation with an ISI of 1 sec*. A) 5% MVC, B) 30% MVC and C) 50% MVC contraction during electrical stimulation with an ISI of 1 sec. The amplitude of each H-reflex was normalized to that of the H-reflex elicited by the first stimulus. Figures on the right top are traces of H-reflexes where surface electromyography (SEMG) responses were rectified and smoothed by 0.1 secs only for subjects to clearly reach the required level of MVC but not to calculate peak-to-peak amplitude of H-reflex. Figures on the right bottom shows the comparison of the H-reflex levels with before contraction average and instant release (¶) following contraction. Error bars show the standard deviation, * $p < 0.05$, N=7. Blue dashed lines show the control H-reflex level evoked by the first stimulus and the black dashed lines show the aimed contraction levels as % MVC.*

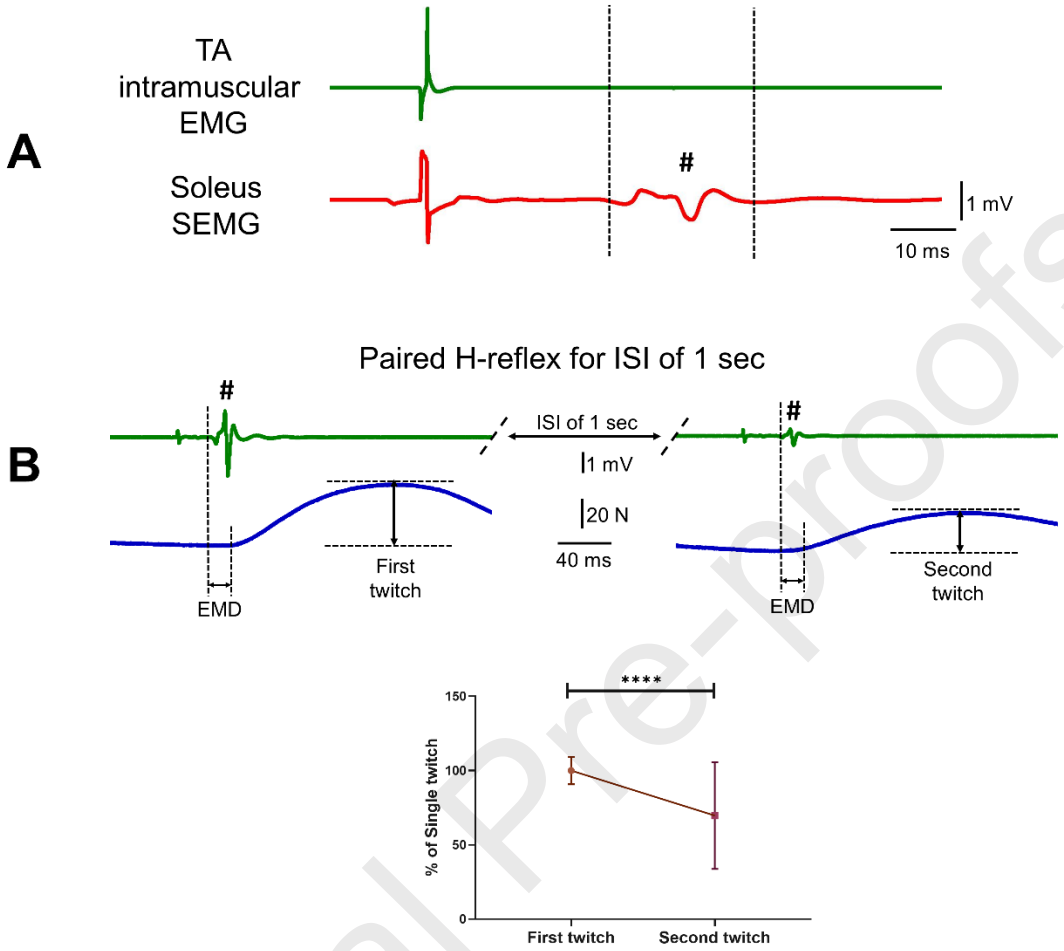

Figure 5. Effect of P-AD on force generation. *A) Sample recordings of TA intramuscular EMG and soleus SEMG are shown. B) Force recording after single and paired H-reflex stimulation with an ISI of 1 sec. "#" symbols indicate the H-reflex. Error bars are the standard deviation. **** $p < 0.0001$ and N=6.*

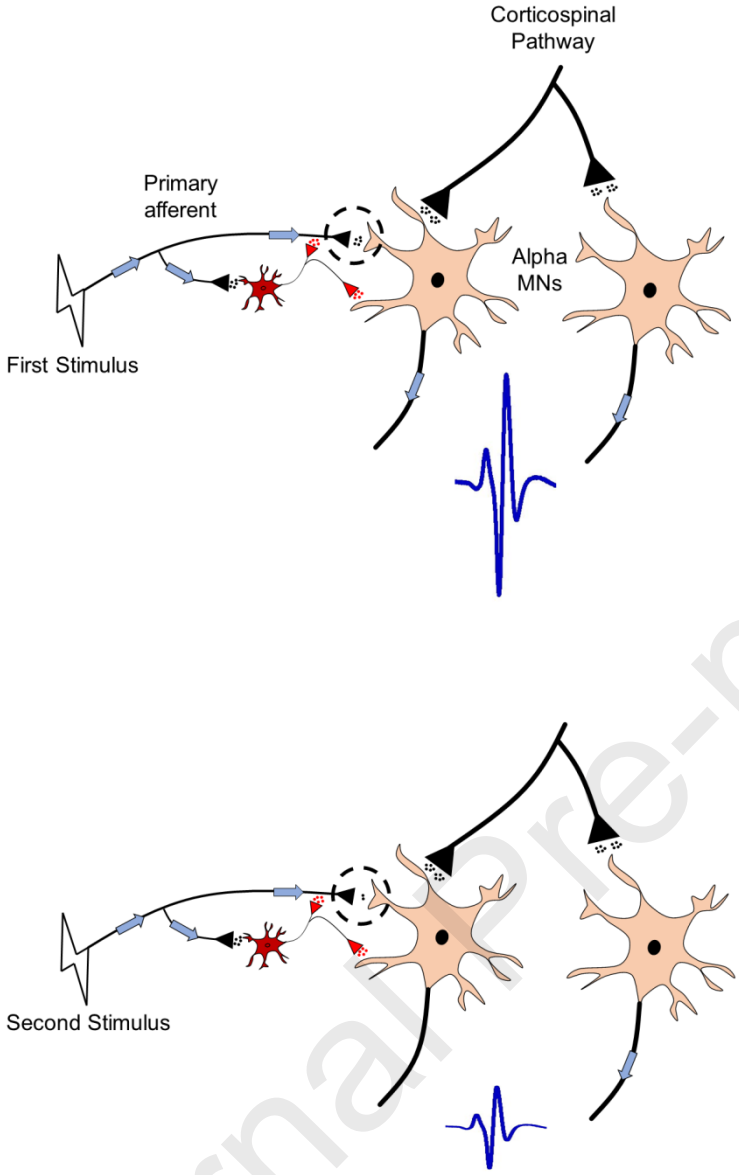

Figure 6. A possible mechanism of P-AD. *The first stimulus might act as an "ON" signal for P-AD and following responses might be shunted by a hypothesized inhibitory interneuron pool (plateau potential interneuron?), for around 5-10 secs. This depression would reduce the number of alpha motoneurons (MN) recruited by primary afferents.*

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Murat Necdet Yüce

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Kemal S. Türker