Available online at www.sciencedirect.com

ScienceDirect

Biomedical Journal

journal homepage: www.elsevier.com/locate/bj

Original Article

Novel human models for elucidating mechanisms of rate-sensitive H-reflex depression

Ya-Ju Chang [a,b], *Yu-Ching Liu* [a,c], *Miao-Ju Hsu* [d,e], *Chia-Ying Fang* [a], *Alice M. Wong* [a,f], *Stacey L. DeJong* [g], *Richard K. Shields* [g,*]

[a] *Department of Physical Therapy and Graduate Institute of Rehabilitation Science, College of Medicine and Healthy Aging Research Center, Chang Gung University, Taoyuan, Taiwan*
[b] *Neuroscience Research Center, Chang Gung Memorial Hospital at Linkou, Taoyuan, Taiwan*
[c] *Department of Physical Medicine and Rehabilitation, Cheng-Hsin General Hospital, Taipei, Taiwan*
[d] *Department of Physical Therapy, College of Health Science, Kaohsiung Medical University, Kaohsiung, Taiwan*
[e] *Department of Physical Medicine and Rehabilitation, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan*
[f] *Department of Physical Medicine and Rehabilitation, Chang Gung Memorial Hospital at Taoyuan, Taoyuan, Taiwan*
[g] *Department of Physical Therapy and Rehabilitation Science, Carver College of Medicine, University of Iowa, IA, USA*

ARTICLE INFO

Article history:
Received 25 April 2018
Accepted 10 July 2019
Available online 26 February 2020

Keywords:
H-reflex
Motor evoked potential
Pre-synaptic
Soleus
Spinal cord
Spinal cord injury

ABSTRACT

Background: This study used novel human neurophysiologic models to investigate whether the mechanism of rate-sensitive H-reflex depression lies in the pre-synaptic or post-synaptic locus in humans. We hypothesized that pre-synaptic inhibition would suppress Ia afferents and H-reflexes without suppressing alpha motor neurons or motor evoked potentials (MEPs). In contrast, post-synaptic inhibition would suppress alpha motor neurons, thereby reducing H-reflexes and MEPs.
Methods: We recruited 23 healthy adults with typical rate-sensitive H-reflex depression, 2 participants with acute sensory-impaired spinal cord injury (SCI) (to rule out influence of sensory stimulation on supra-spinal excitability), and an atypical cohort of 5 healthy adults without rate-sensitive depression. After a single electrical stimulation to the tibial nerve, we administered either a testing H-reflex or a testing MEP at 50–5000 ms intervals.
Results: Testing MEPs were not diminished in healthy subjects with or without typical rate-sensitive H-reflex depression, or in subjects with sensory-impaired SCI. MEP responses were similar in healthy subjects with versus without rate-sensitive H-reflex depression.
Conclusions: Results from these novel *in vivo* human models support a pre-synaptic locus of rate-sensitive H-reflex depression for the first time in humans. Spinal reflex excitability can be modulated separately from descending corticospinal influence. Each represents a potential target for neuromodulatory intervention.

* *Corresponding author*. Department of Physical Therapy and Rehabilitation Science, Carver College of Medicine, University of Iowa, 1-252 Medical Education Building, 375 Newton Rd, Iowa City, IA, 52242, USA.
E-mail address: richard-shields@uiowa.edu (R.K. Shields).
Peer review under responsibility of Chang Gung University.
https://doi.org/10.1016/j.bj.2019.07.007

At a glance of commentary

Scientific background on the subject

We used a novel human neurophysiologic model to understand if segmental rate-sensitive H-reflex depression occurs in the pre-synaptic or post-synaptic locus in humans. The novel experiments in humans elucidated a pre-synaptic locus as the underlying mechanism for the rate-sensitive H-reflex depression that is observed in humans.

What this study adds to the field

The novel methods from this study shows that we can non-invasively differentiate between pre-synaptic and post-synaptic inhibitory mechanisms in humans. Our ability to differentiate these mechanisms raises the possibility to develop novel rehabilitation strategies to impact neuromodulation and understand the neuroplasticity of these pathways in humans.

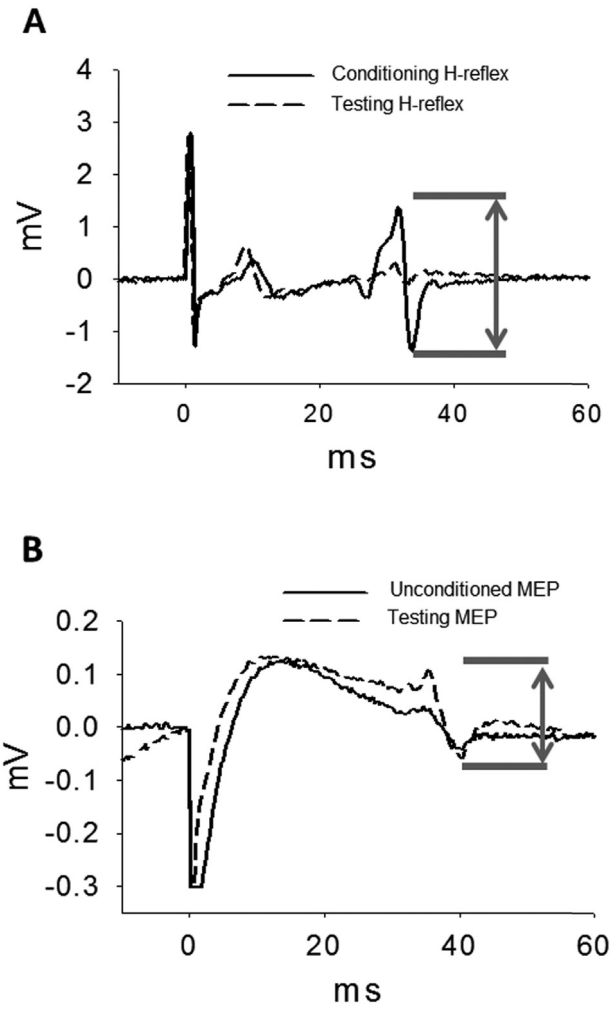


Fig. 1 **Representative examples from one healthy subject in Group I.** Soleus EMG recordings following (A) conditioning and testing H reflexes; and (B) conditioning and testing MEPs. The amplitude of the H reflex and MEP were calculated as the peak-to-peak difference (arrows).

People with spinal cord injury (SCI) and other neurological conditions often have increased excitability of monosynaptic spinal reflex circuits, which manifests as spasticity, contributes to impaired motor performance, and can be measured using the H-reflex response to peripheral nerve stimulation. In healthy humans [1–7] and animals [1,8–10], amplitude of the H-reflex demonstrates rate-sensitive depression during repetitive stimulation. An initial electrically induced discharge of Ia afferent fibers exerts an inhibitory influence that diminishes subsequent H-reflex responses. A reduction in rate-sensitive depression seems to be one of the mechanisms underlying spasticity in patients with SCI or hemiplegia [2,6,11–14]. The amount of H-reflex depression observed in individuals with acute SCI (less than six months post injury) is similar to that in neurologically-intact humans, but rate-sensitive depression appears to wane as clinically observable spasticity develops [2,7,15,16]. The apparent link between rate-sensitive depression and clinical spasticity has triggered extensive research into the possible shared mechanisms underlying these two phenomena.

Rate-sensitive depression of H-reflexes is assessed by delivering an initial electrical stimulus to a peripheral nerve, followed by a test stimulus to the same nerve. The initial conditioning stimulus elicits an efferent response (M-wave) while concurrently activating the Ia afferent/alpha motor neuron reflex arc, yielding an H-reflex [Fig. 1A]. Activation of the Ia afferents also excites spinal inhibitory interneurons, which either directly inhibit the alpha motor neuron (post-synaptic mechanism) or they inhibit the pre-synaptic terminal of the Ia afferent, effectively reducing the likelihood of activation of the Ia/alpha reflex arc during subsequent electrical stimulation. In the healthy condition, rate-sensitive depression may help sustain the synaptic efficacy of the Ia fiber at a relatively low level during voluntary movements by maintaining a low gain for the stretch reflex; functionally, this may help prevent clonus from developing [12–14,17–19].

Historically, two lines of thought have emerged regarding the neurophysiologic substrate for rate-sensitive depression. Both hinge upon the effects of inhibitory interneurons that modulate the Ia/alpha motoneuron arc. Many investigators believe that the Ia afferent receives this inhibition pre-synaptically [5,8,16,20,21]. Other studies have suggested that inhibitory interneurons exert their influence post-synaptically [6,22,23]. Some researchers attribute this to the homonymous presynaptic inhibition of Ia afferents (in humans) [5,12,16,21,24–26], but heteronymous presynaptic inhibition strategies contribute at various latencies [27]. Others suggest that post-synaptic Renshaw cell inhibition also contributes to the depression of the H-reflex at an interval shorter than 110 ms [26], with the majority of evidence implicating latencies shorter than 40 ms [23,24]. In the cat, researchers have observed depression of the excitatory post-

synaptic potential (EPSP) originating from the previously-activated Ia afferents without accompanying depression of Ia afferent fiber EPSPs from other heteronymous nerves [12,13,28]. In humans, the motor evoked potential (MEP) is not depressed at 2 s after passive stretch, suggesting that post-synaptic inhibition of the motor neurons is not the mechanism [12]. Although most investigators today believe the most likely mechanism of rate-sensitive depression is pre-synaptic inhibition, post-synaptic mechanisms have not been definitively ruled out in various latencies, particularly in humans.

Combining peripheral electrical stimulation with transcranial magnetic stimulation (TMS) can provide unique insight into the neurophysiologic basis of rate-sensitive depression. Because the MEP is not mediated by Ia afferent neurons, it can be evoked regardless of the excitation state of the pre-synaptic Ia afferent terminal. If interneurons exert their influence via pre-synaptic pathways, then the MEP amplitude should be unaffected by a prior peripheral electrical stimulus. However, if interneurons exert their influence via post-synaptic inhibition of alpha motoneurons, then the MEP amplitude should be decreased [Fig. 2]. This technique offers a method to perform a pathway analysis in humans.

A potential difficulty to studying human rate-sensitive depression with this technique is that supra-spinal excitability may be influenced by afferent volleys initiated by the conditioning stimulation. Previous studies found that the activity of pyramidal tract neurons in the primate motor cortex changed in response to peripheral stimulation [29–31]. In humans, the MEP is changed following peripheral nerve stimulation [32–35,40]. However, since the amplitude of the MEP reflects the sum of excitability of the entire motor pathway, facilitation at the motor cortex level combined with inhibition at the segmental post-synaptic level may yield unchanged MEPs.

To avoid this potential problem, animal studies routinely ablate afferent pathways in order to isolate segmental responses from afferent feedback loops. This is of course impossible in human studies, but individuals with incomplete SCI (American Spinal Injury Association Impairment Scale, AIS class C or D) with sensory impairment are a novel alternative model. In a previous study, we found that peripheral stimulation did not influence supra-spinal excitability in individuals with incomplete SCI, supporting that sensory pathway disruption served to isolate efferent responses from ascending afferent influences [36]. Thus, in this type of subject, changes of the MEP can reveal isolated excitability changes at segmental post-synaptic structures in the absence of confounding afferent factors [Fig. 2B].

A second novel human model for studying the effect of peripheral stimulation on the excitability of supra-segmental structures in neurologically-intact subjects who do not demonstrate the typical rate-sensitive depression of the H-reflex [4,37]. These individuals do not demonstrate segmental inhibition of the Ia/alpha motoneuron arc; thus any changes to an MEP after a conditioning stimulus can be assumed to reflect ascending afferent influence on supra-spinal excitability [Fig. 2C].

The purpose of this study was to use novel human neurophysiologic models to determine whether the locus of H-reflex depression in humans lies on the pre- or post-synaptic terminals. To examine the influence of sensory stimulation on supra-spinal excitability, we recruited participants with acute sensory-impaired SCI and neurologically healthy subjects who did not demonstrate rate-sensitive depression. If the mechanism for rate sensitive depression of the H-reflex acts at the pre-synaptic level, the conditioned MEP will show no depression in any subject groups [Fig. 2D]. If, however, the mechanism is post-synaptic, yielding direct inhibition of the alpha motor neuron, MEPs may be depressed in healthy subjects with typical rate-sensitive depression and will be depressed in subjects with SCI. In participants who do not normally show rate-sensitive depression, and who therefore lack typical spinal inhibitory interneuron contributions, the MEP will not be depressed. In this case, if the mechanism is post-synaptic, MEPs would be relatively facilitated compared to subjects with rate-sensitive H-reflex depression and subjects with SCI [Fig. 2].

Material and Methods

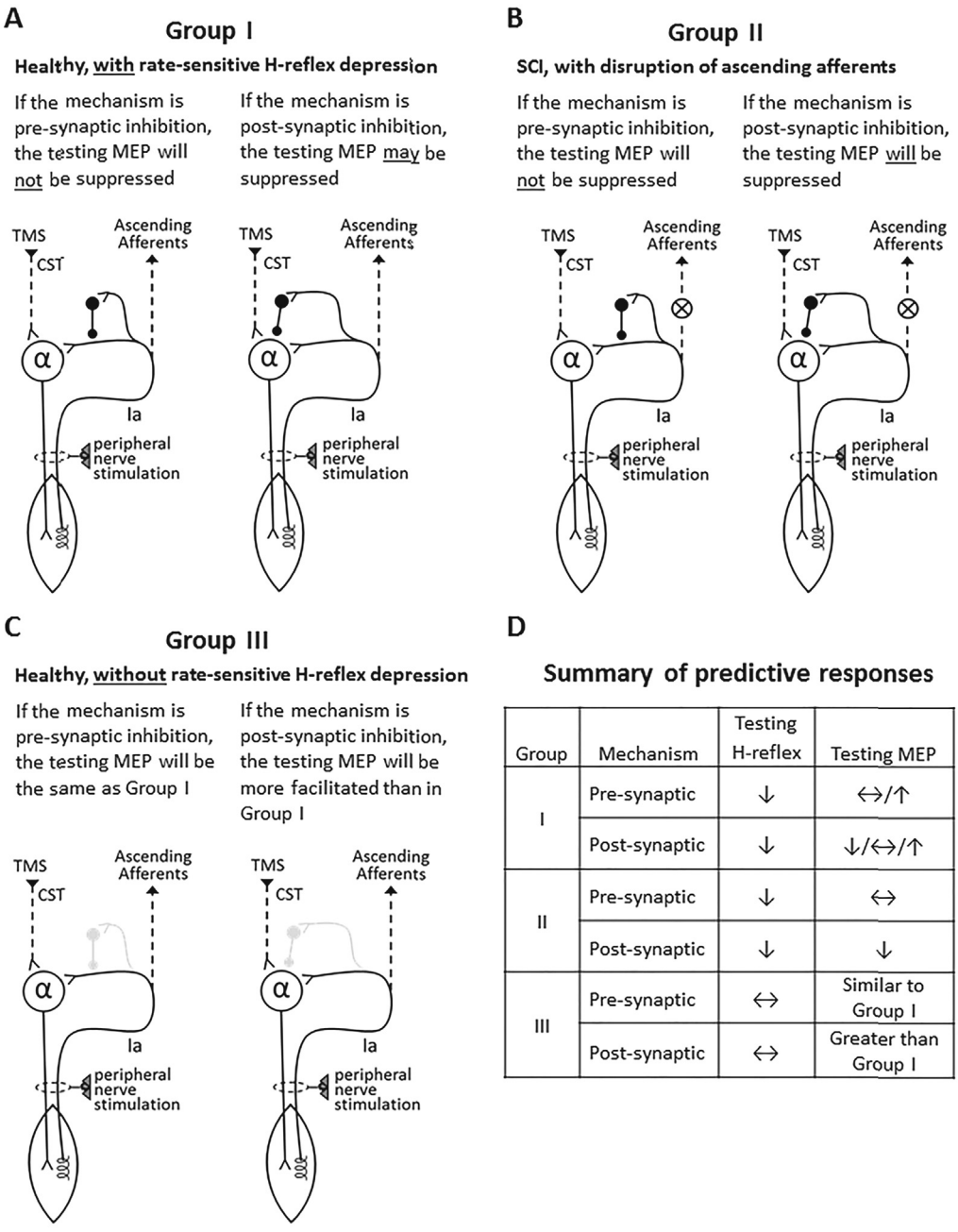
Subjects

Thirty individuals were recruited as three groups. Group I included 23 healthy individuals (11 male 12 female, aged 22.22 ± 3.15 years) who had no physical disabilities and who showed rate-sensitive depression of the soleus H-reflex. Group II included 2 individuals (male, aged 24 and 30 years old) with acute incomplete spinal cord injury of less than six months' duration. Group III included 5 healthy individuals (3 male, 2 female, aged 22.00 ± 2.19 years) who had no physical disabilities but did not show rate-sensitive depression of the soleus H-reflex [Table 1]. Subjects in Group I and Group III had no previous history of neuro-musculoskeletal disease and had no lower extremity injuries within two years prior to testing. Subjects were *a posteriori* assigned to Group III if they did not demonstrate rate-sensitive depression during the experimental protocol. Subjects in Group II had incomplete SCI confirmed by a neurological examination, indicating preserved motor function but impaired sensory function (absent light touch, sharp-dull, two point discrimination, proprioception, and kinesthesia) below the level of injury. The subjects could voluntarily contract the soleus muscle to produce ankle plantar flexion through at least half of the full range of motion. All subjects provided written informed consent in accordance with the Declaration of Helsinki. The study was approved by the institution's human subjects review board.

Measurements were made from one randomly selected leg for each subject. The subject was seated comfortably in a wheelchair with the ankle dorsiflexed to a neutral position and the knee flexed 70° from full extension. The foot was secured to a fixation footplate to ensure that muscle contractions were isometric.

Electromyography recordings

Surface EMG signals (H-reflexes, M-waves, and MEPs) were recorded using 8-mm-diameter bipolar silver-silver chloride electrodes with 20 mm fixed inter-electrode distance (B&L Engineering, Canada). The recording electrode was positioned in



Group	Mechanism	Testing H-reflex	Testing MEP
I	Pre-synaptic	↓	↔/↑
	Post-synaptic	↓	↓/↔/↑
II	Pre-synaptic	↓	↔
	Post-synaptic	↓	↓
III	Pre-synaptic	↔	Similar to Group I
	Post-synaptic	↔	Greater than Group I

Fig. 2 **Illustration of the hypotheses.** Panes A, B, and C show schematic diagrams of hypothesized pre-synaptic (left) and post-synaptic (right) neural circuits. V-shaped connections indicate excitatory synapses and black circles represent inhibition. Abbreviations: TMS, transcranial magnetic stimulation; CST, corticospinal tract. (A) In healthy subjects with rate-sensitive depression of the H-reflex, if the depression is at the presynaptic level, the testing MEP will not be suppressed because it does not depend upon the excitation status of the pre-synaptic Ia terminal. However, if the depression is at the postsynaptic level, the testing MEP may or may not be suppressed, depending on the degree of facilitation from afferent stimulation. (B) In individuals with acute SCI who have intact motor pathways but disrupted ascending afferents (shown as an X), if the depression is at the presynaptic level, the testing MEP will not be suppressed. However, if the depression is at the postsynaptic level, the testing MEP will be suppressed. (C) In healthy individuals without rate-sensitive depression of H-reflex, if the depression is at the presynaptic level, the testing MEP will be similar to those with rate-sensitive depression of the H-reflex. However, if the depression is at the postsynaptic level, the testing MEP will not show suppression and will be facilitated more than individuals with rate-sensitive depression of H-reflex. (D) Summary of the predictive responses of the testing H reflex and MEP in the three groups.

Table 1 Subject demographics.

	Group I	Group II	Group III
Number	23	2	5
Sex	11 M/12 F	2 M	3 M/2 F
Age (yr)[a]	22.2 ± 3.15	27.0 ± 4.24	22.0 ± −2.19
Height (cm)[a]	165.8 ± 6.84	166.5 ± 2.12	168.2 ± 8.59
Weight (kg)[a]	58.7 ± 11.46	60.0 ± 0	66.6 ± 11.67
Time post SCI (months)[a]		2.5 ± 1.4	
Level of Injury		C3, L4	
AIS Classification		D, D	

Abbreviations: M:male; F:female.
[a] Mean ± 1 SD.

parallel with the soleus muscle, approximately 2 cm medial to the midline of the distal calf and distal to the medial head of the gastrocnemius. A ground electrode was placed anteriorly over the tibia. Each electrode contained an on-site pre-amplifier with a gain of 350. The signal was amplified further by a mainframe amplifier (Gould 335, Gould Instrument System Inc, USA) with adjustable gain from 0 to 500. The amplifier utilized a high impedance circuit, greater than 11.6 MΩ, with a common mode rejection ratio of 87 dB at 60 Hz and a bandwidth of DC-4000 Hz.

Electrical stimulation

H-reflexes and M-waves of the soleus muscle were elicited by electrical stimulation of the tibial nerve using a constant-current stimulator (Digitimer DS7A, Digitimer Ltd, Welwyn Court, UK) with a range of 100–400 V and a constant current up to 1000 milliamps. The stimulator was triggered by custom-written software. The tibial nerve was stimulated in the popliteal fossa, with the cathode placed over the tibial nerve and the anode placed over the patella. At the beginning of each experiment, optimal location of the cathode was determined by adjusting its position manually, while stimuli were delivered at approximately 5 s intervals and responses were displayed on a monitor. With the cathode in its initial location, stimulus intensity was gradually increased until H-reflexes and M-waves were observed, then the cathode was moved medially and laterally to identify the location resulting in the largest response amplitudes. The cathode was then secured in that location with adhesive tape to ensure stationary positioning throughout the experiment. The stimulus intensity necessary to elicit a maximal M-wave of the soleus was determined using a pulse width of 200 μs and gradually increasing stimulation intensity until no further increase in peak-to-peak amplitude was observed. A supramaximal stimulus was then delivered (1.2 times the intensity required to produce the largest M-wave), to determine the maximal M-wave amplitude (Mmax). For eliciting H-reflex of the soleus, the pulse width was 1000 μs Stimulation intensity was gradually adjusted until it elicited an H-reflex with amplitude between 20% and 50% of Mmax, on the ascending limb of the H-reflex recruitment curve.

Magnetic stimulation

MEPs in the relaxed soleus were obtained by stimulating the scalp using a transcranial magnetic stimulator (Magstim 200 Magstim Company Ltd, UK) and a figure-of-eight focal coil (10 cm external wing diameter). The vertex was identified and marked on a cap. The center of the coil was placed on the scalp just lateral to the vertex on the side contralateral to the recorded soleus. The best location for delivering TMS in each subject was determined by moving the coil by 1 cm increments in each direction. The location consistently producing the largest MEPs at the lowest intensity was marked and selected as the site for TMS for the remainder of the experiment. The coil was fixed on a custom-made fixation frame so that the position and orientation of the coil were kept constant throughout the experiment. The resting motor threshold (rMT) of MEP was taken as the lowest intensity that elicited 5 MEPs of greater than 50 μV out of 10 stimulations. The stimulation intensity for the remainder of the experiment was adjusted to 120% rMT.

Procedure

For normalizing purposes, five maximum M-waves, five unconditioned H-reflexes, and five unconditioned MEPs were recorded as baseline data. The maximal M-waves, unconditioned H-reflexes, and unconditioned MEPs were repeated and assessed visually in the middle and at the end of the experiment to verify that the recording condition was constant.

Subjects then received paired electrical peripheral stimulations, and electrical peripheral stimulation paired with TMS. In the paired electrical peripheral stimulation, the tibial nerve was stimulated twice at 50 ms, 100 ms, 133 ms, 200 ms, 400 ms, 1000 ms, and 5000 ms intervals to elicit two H-reflexes. The first stimulus was called a conditioning stimulation and the second H-reflex was called a testing H-reflex. The testing H-reflexes were noted as H_{50}, H_{100}, H_{133}, H_{200}, H_{400}, H_{1000}, and H_{5000}, respectively. In the paired electrical – transcranial stimulations, the tibial nerve was first stimulated to elicit a conditioning H-reflex, and the motor cortex was then stimulated by the transcranial magnetic stimulator 50 ms, 100 ms, 133 ms, 200 ms, 400 ms, 1000 ms, or 5000 ms later to elicit a testing MEP. The testing MEPs were noted as MEP_{50}, MEP_{100}, MEP_{133}, MEP_{200}, MEP_{400}, MEP_{1000}, and MEP_{5000}, respectively. The stimulating pairs were elicited in a randomized order and were repeated five times at each of the intervals. At least 10 s elapsed between each set of paired stimuli. In Group II, the electrical – transcranial stimulation pairs were tested only at 50 ms, 400 ms, and 1000 ms intervals in order to shorten the experiment time for subjects with acute SCI. These three intervals were chosen to assess rate-sensitive H-reflex depression across a range of stimulation frequencies that may correspond to different neurophysiological mechanisms. Whereas classical pre-synaptic inhibition may dominate when the inter-stimulus interval is short, homosynaptic depression may contribute to longer lasting H-reflex depression [5,7,25].

Data analysis

The peak-to-peak amplitude of testing H-reflexes and MEPs [Fig. 1] were normalized to Mmax and then expressed as a percentage of the conditioning H-reflex or the unconditioned MEP. For Group I, we evaluated effects of inter-pulse intervals

on testing H-reflexes and testing MEP amplitudes, using Friedman tests with a significance level of $p < 0.05$. Where indicated, Wilcoxon signed rank tests were used for pairwise comparisons, with a Bonferroni adjusted p-value of $p < 0.007$ to account for multiple comparisons. To test for differences between Groups I and III, we analyzed testing H-reflexes and MEP amplitudes using a Mann Whitney U tests and a Bonferroni adjusted p-value of $p < 0.007$. Because there were only two subjects in Group II, descriptive analysis was used to illustrate changes in the testing H-reflex and the testing MEP.

Results

H-reflex and MEP responses in healthy individuals with rate-sensitive H-reflex depression

For Group 1, the Friedman test showed a significant difference in H-reflex amplitudes across inter-pulse intervals (df = 7, Q = 133.59, $p < 0.0001$). Wilcoxon signed rank tests indicated that the testing H-reflex was significantly less than the conditioning H-reflex at all inter-pulse intervals ($p < 0.007$ for all comparisons). The amount of depression was largest at the 50 ms interval, such that H_{50} was only 9% ± 5% of the conditioning H-reflex. Testing H-reflex depression was lesser at longer intervals, largely vanishing at 5000 ms (H_{5000} = 93% ± 13% of the conditioning H-reflex; steadily progressing from 9% at H_{50} to 93% at H_{5000}).

Unlike the testing H reflex, the testing MEP was not significantly diminished at any interval except 1000 ms (df = 7, Q = 39.06, $p < 0.0001$, Wilcoxon signed rank test $p < 0.0001$ for MEP_{1000}, MEP_{1000} = 65% ± 24% of the unconditioned MEP). There was a trend toward facilitation of the MEP at the 50 ms interval, which did not reach the threshold for significance [Fig. 4] (Wilcoxon signed rank test $p = 0.0232$, MEP_{50} = 163% ± 109% of the conditioning MEP). At all other inter-pulse intervals, the testing MEPs ranged between 78% ± 45% and 129% ± 81% of the unconditioned MEP, and were not significantly different than the unconditioned MEP (Wilcoxon signed rank tests $p > 0.007$) [Fig. 4].

H-reflex and MEP responses in individuals with incomplete SCI

In Group II, the pattern of the testing H-reflex was similar to that in Group I. The H_{50}, H_{100}, H_{133}, H_{200}, H_{400}, and H_{1000} were depressed to 5% ± 5%, 34% ± 46%, 39% ± 51%, 47% ± 36%, 78% ± 27%, and 52% ± 51% of the conditioning H-reflex, respectively [Fig. 3]. The H_{5000} recovered to 83% ± 20% at the 5000 ms interval. As observed for most inter-pulse intervals in Group I, testing MEP amplitude for Group II did not differ substantially from the unconditioned MEP [Fig. 4]. Descriptively, the amplitudes of MEP_{50}, MEP_{400}, and MEP_{1000} were 105%, 160% and 143% of the unconditioned MEP respectively in one subject with SCI, and 107%, 109% and 115% respectively in the other. Although in Group 1, MEP_{1000} demonstrated significant depression (65% of the unconditioned MEP), that did not occur in Group II.

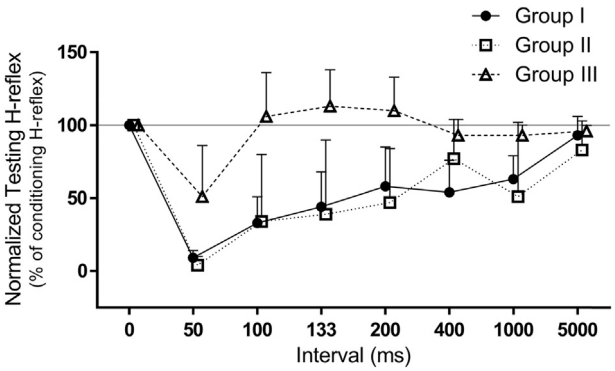


Fig. 3 **Normalized testing H-reflexes.** Testing H-reflexes, expressed as a percent of the conditioning H-reflex, for different stimulation intervals in healthy subjects with rate-sensitive depression of the H-reflex (Group I), subjects with SCI and disrupted ascending afferents (Group II), and healthy subjects without rate-sensitive depression of the H-reflex (Group III). Interval 0 indicates the conditioning H-reflex. The grey line at 100% is provided for reference. Error bars represent 1 SD. The testing H-reflex was significantly diminished at all intervals in Group I, and the pattern of H-reflex depression was similar in Group II. In Group III, the testing H-reflex was not significantly different from the conditioning H-reflex at any interval, although inhibition at the 50 ms interval approached significance ($p = 0.0625$).

H-reflex and MEP responses in individuals without rate-sensitive depression

In Group III, although the Friedman test indicated significant effects of inter-pulse interval on H-reflexes ($p = 0.0377$) and

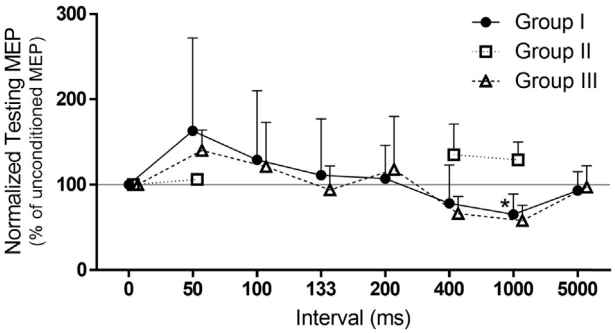


Fig. 4 **Normalized testing MEPs.** Testing MEPs, expressed as a percent of the unconditioned MEP, for different stimulation intervals in healthy subjects with rate-sensitive depression of the H-reflex (Group I), subjects with SCI and disrupted ascending afferents (Group II), and healthy subjects without rate-sensitive depression of the H-reflex (Group III). Interval 0 indicates the unconditioned MEP. The grey line at 100% is provided for reference. Error bars represent 1 SD. * In Group I, the testing MEP was significantly decreased at the 1000 ms interval ($p < 0.0001$). MEP facilitation at the 50 ms interval approached significance ($p = 0.0232$). The testing MEP was unchanged at all other intervals. In Group II, the testing MEP did not differ substantially from the unconditioned MEP. In Group III, the pattern of change of the testing MEP was similar to that in Group I.

MEPs ($p = 0.0044$), pairwise comparisons using Wilcoxon signed rank tests revealed no significant depression or facilitation at any of the inter-pulse intervals (all $p > 0.007$). There was a trend toward depression of the H-reflex at the 50 ms interval, which did not reach the threshold for significance [Fig. 3] ($p = 0.0625$, $H_{50} = 51\% \pm 35\%$ of the conditioning H reflex).

In Group III, the effect of inter-pulse interval on the testing MEP was similar to that in Group I [Fig. 4]. The Mann Whitney U test showed no significant difference between Group 1 and Group III in the amplitude of conditioned MEPs at any of the inter-pulse intervals (all $p > 0.007$). These results suggested that the MEPs of Group III were not facilitated more than Group I.

Discussion

Significance of the findings

In healthy subjects with rate-sensitive depression of H-reflex (Group I), the testing H-reflex was depressed at stimulation intervals between 50 ms and 5 s. The testing MEP was not depressed at the same stimulation intervals, indicating that the efferent pathway, including the alpha motoneuron, was not under inhibitory influence. This suggests that the inhibitory mechanisms that caused H-reflex rate-sensitive depression acted pre-synaptically, not post-synaptically. Likewise, individuals with acute incomplete SCI (Group II) demonstrated non-suppressed MEPs that implicate pre-synaptic mechanisms. Finally, MEP suppression in healthy subjects without rate-sensitive depression (Group III) did not differ from Group I, providing additional evidence for pre-synaptic mechanisms [Fig. 2].

In healthy subjects with rate-sensitive depression of the H-reflex, the amount of H-reflex depression elicited by paired electrical stimulation was comparable to that reported by previous researchers using paired stimulation protocols [3,5,38]. Kagamihara et al. [38] found that the testing H-reflex was initially recovered to 60% of the conditioning H-reflex between 200 ms and 300 ms, and further recovered after 800 ms. Our results indicated that the testing H-reflex recovered to $57 \pm 27\%$ at 200 ms. At longer stimulation intervals, our study found that the H_{1000} and H_{5000} depressed to $63 \pm 16\%$ and $93 \pm 13\%$ of the control, respectively. These amounts of inhibition are similar to those reported by Kohn et al. [5] which were 53% and 85%, respectively. Sarmadi et al. [26] reported a higher percentage of recovery (70%) at 200 ms. However, in the study by Sarmadi et al. [26], the interval between the two stimulation pairs was 2.5 s, much shorter than that used in our study. A short inter-stimulation- pair interval will not allow full recovery of the following conditioning H-reflex [39], resulting in less depression.

Although our study is not the first to suggest that the mechanism for rate sensitive depression of the H-reflex occurs at the pre-synaptic level in humans, our study is the first to provide evidence across a wide time spectrum. Kohn et al. [5] reported that the MEP did not suppress at 1 s following a conditioning stimulation. Hultborn et al. [12] found that the MEP did not depress 2 s after passive stretching of the soleus. Because rate sensitive depression of H reflex has been described at stimulus intervals as long as 10 s [10], our experimental protocol included inter-pulse intervals spanning 50 ms to 5 s. The similarity between MEP suppression in our protocol and during passive stretch [12] supports that similar neurophysiologic processes underlie post-activation depression in both conditioning modes (stimulation versus stretch).

Our study is the first to use mechanistically-grounded human models to examine the neurophysiologic basis of rate-sensitive depression *in vivo*. Testing individuals with acute incomplete SCI (Group II) allowed us to isolate MEP responses from afferent feedback pathways that could alter supra-segmental excitability of the testing MEP. In subjects with sensory-impaired SCI, ascending afferent potentials elicited by the conditioning H-reflex are disrupted at the lesion site, blocking transmission to supra-spinal structures. Thus, changes of the testing MEPs reflect changes in excitability of efferent structures, particularly post-synaptic structures. These subjects showed depression of the testing H-reflex (intact segmental inhibition of the Ia/alpha motor neuron reflex arc) but no depression of the testing MEP (no evidence for direct post-synaptic segmental inhibition upon the alpha motoneuron). A key feature of these participants was that because their SCI was recent (<6 months), they demonstrated intact H-reflex rate-sensitive depression. With increasing time post-SCI, this depression would be expected to wane [7]. However, this phenomenon has only been studied in participants with motor and sensory complete (AIS A) SCI. Future studies are needed to confirm that H-reflex rate-sensitive depression is likewise lost in patients with AIS C or D SCI. The preservation of volitional efferent volleys within the spinal circuitry could foreseeably alter the manifestation of this change in patients with sensory-incomplete SCI.

Findings from Group III (healthy subjects without rate-sensitive depression) also implicate a pre-synaptic mechanism for rate-sensitive depression of the H-reflex. As outlined in Fig. 2, if a post-synaptic mechanism was involved, the testing MEP should have been relatively facilitated compared to MEPs observed in Group I participants. The equivalence of MEP modulation between Group I and Group III supports a pre-synaptic, rather than a post-synaptic mechanism. Interestingly, in Group III, inhibition of the testing H-reflex approached significance at the 50 ms inter-pulse interval, while no inhibition was apparent at the other intervals [Fig. 3]. A plausible speculation is that the mechanisms for H reflex depression at 50 ms are different from those at 100 ms or longer. Recurrent inhibition, Ib inhibition and refractoriness following after-hyperpolarization in motoneurons are well known factors that may affect reflex responses at short conditioning intervals. Sarmadi et al. [26] argued that Renshaw cell inhibition could persist up to 110 ms, but other researchers believed that the influence was much shorter [23,24]. It is possible that mechanisms such as long-lasting decrease of the motoneurons excitability caused by the activation of spinal cord interneurons (Renshaw, Ib, and others) or by motoneuron dynamics (after-hyperpolarization), influenced the depression of the testing H reflex at intervals shorter than 50 ms. However, since the testing MEP_{50} for participants with incomplete SCI

was not depressed, any active mechanisms were likely to be pre-synaptic rather than post-synaptic.

Other possible mechanisms for depression of the testing H-reflex at 50 ms may involve the Ia afferent fiber. Activity-dependent action potential propagation failure at Ia axonal branch points following repetitive stimulation might contribute to the inhibition of the testing H-reflex within 1 s [41]. Classic GABAergic pre-synaptic inhibition of Ia afferent terminals might also play a role. Previous researchers have observed effects of classical pre-synaptic inhibition at conditioning-test intervals from 20 ms to 400 ms [42,43]. In our protocol, the partial maintenance of H-reflex depression up to 5000 ms implicates mechanisms distinct from classical pre-synaptic inhibition and action potential propagation failure at the Ia axonal branch. As discussed previously, the mechanism for rate-sensitive depression, especially for intervals longer than 1 s, appears to be similar to that for post-activation depression [12]. Post-activation inhibition has been suggested to reflect different processes from classic pre-synaptic inhibition (e.g. depletion of the neurotransmitter [5] of the homosynaptic interneuron connections [12]).

Methodological considerations

Participants assigned to Group III were all healthy without previous history of neuromuscular disease. This novel sub-population has been captured rarely in previous studies, perhaps because they do not exhibit any noteworthy functional or clinical characteristics. The neurophysiologic basis for the absence of rate-sensitive depression in these participants is not known and is beyond the scope of the present study.

Without active inhibitory influences upon the Ia/alpha motoneuron reflex arc, one may question whether Group III participants could fully relax the soleus muscle. If subjects in Group III showed greater baseline soleus activity due to lost segmental inhibition, the MEP would show facilitation. The equivalence of MEP amplitudes between Group III and Group I supports that this was not the case. In addition, we visually monitored the EMG of the soleus and the tibialis anterior, but observed no evidence of muscle contraction. Thus the absence of H reflex depression for Group III could not be explained by differences in baseline EMG activity.

Conclusions

Two novel *in vivo* human models and a combined H-reflex/MEP test protocol offered evidence for a pre-synaptic mechanism for rate-sensitive H-reflex depression. MEP_{50} facilitation in neurologically-intact humans (Groups I and III) but not in participants with sensory-impaired SCI (Group II) supports a supra-segmental locus for MEP facilitation with short interpulse intervals. The method used in this study enabled us to non-invasively differentiate between pre-synaptic and post-synaptic inhibitory mechanisms in humans. Efforts are underway to develop novel rehabilitation strategies, which aim to modify excitability of neural circuitry by inducing targeted neuroplasticity [44]. Findings from the current study suggest that it may be possible to impact spinal segmental circuits and corticospinal circuits separately. Therefore both may be suitable targets for neuromodulation, and potential benefits may be additive.

Funding

This work was supported by the Ministry of Science and Technology, Taiwan [grant number MOST 105-2918-I-182-002], 107-2221-E-182-009-MY3; Healthy Aging Research Center at Chang Gung University [grant number EMRPD1I0501]; Chang Gung Medical Foundation [grant number CMRPD3E0112]; the Neuroscience Research Center, Chang Gung Memorial Hospital, Linkou Medical Center in Taiwan; and by the United States National Institutes of Health [grant numbers R01-HD084645, R01-HD082109 and K12-HD055931]. The funding sources had no involvement in the collection, analysis or interpretation of data, in the writing of the report, or in the decision to submit the article for publication.

Conflicts of interest

The authors declare that they have no competing interests.

Acknowledgements

The authors acknowledge Dr. Shauna Dudley-Javoroski for her careful review of this manuscript.

REFERENCES

[1] Andrews JC, Stein RB, Roy FD. Post-activation depression in the human soleus muscle using peripheral nerve and transcutaneous spinal stimulation. Neurosci Lett 2015;589:144–9.
[2] Chang YJ, Liang JN, Hsu MJ, Lien HY, Fang CY, Lin CH. Effects of continuous passive motion on reversing the adapted spinal circuit in humans with chronic spinal cord injury. Arch Phys Med Rehabil 2013;94:822–8.
[3] Crone C, Nielsen J. Methodological implications of the post activation depression of the soleus H-reflex in man. Exp Brain Res 1989;78:28–32.
[4] Ishikawa K, Ott K, Porter RW, Stuart D. Low frequency depression of the H wave in normal and spinal man. Exp Neurol 1966;15:140–56.
[5] Kohn AF, Floeter MK, Hallett M. Presynaptic inhibition compared with homosynaptic depression as an explanation for soleus H-reflex depression in humans. Exp Brain Res 1997;116:375–80.
[6] Pierrot-Deseilligny E, Burke D. Circuitry of the human spinal cord: spinal and corticospinal mechanisms of movement. Circuitry of the human spinal cord: spinal and corticospinal mechanisms of movement. 2012. p. 1–606.
[7] Schindler-Ivens S, Shields RK. Low frequency depression of H-reflexes in humans with acute and chronic spinal-cord injury. Exp Brain Res 2000;133:233–41.
[8] Caron G, Marqueste T, Decherchi P. Restoration of post-activation depression of the H-reflex by treadmill exercise in aged rats. Neurobiol Aging 2016;42:61–8.

[9] Lee-Kubli CA, Calcutt NA. Altered rate-dependent depression of the spinal H-reflex as an indicator of spinal disinhibition in models of neuropathic pain. Pain 2014;155:250–60.
[10] Lloyd DP, Wilson VJ. Reflex depression in rhythmically active monosynaptic reflex pathways. J Gen Physiol 1957;40:409–26.
[11] Fang CY, Hsu MJ, Chen CC, Cheng HYK, Chou CC, Chang YJ. Robot-assisted passive exercise for ankle hypertonia in individuals with chronic spinal cord injury. J Med Biol Eng 2015;35:464–72.
[12] Hultborn H, Illert M, Nielsen J, Paul A, Ballegaard M, Wiese H. On the mechanism of the post-activation depression of the H-reflex in human subjects. Exp Brain Res 1996;108:450–62.
[13] Nielsen JB, Crone C, Hultborn H. The spinal pathophysiology of spasticity–from a basic science point of view. Acta Physiol (Oxf) 2007;189:171–80.
[14] Smith AC, Knikou M. A review on locomotor training after spinal cord injury: reorganization of spinal neuronal circuits and recovery of motor function. Neural Plast 2016;2016:1216258.
[15] Ditunno JF, Little JW, Tessler A, Burns AS. Spinal shock revisited: a four-phase model. Spinal Cord 2004;42:383–95.
[16] Oza PD, Dudley-Javoroski S, Shields RK. Dynamic fatigue does not alter soleus H-reflexes conditioned by homonymous or heteronymous pathways. Mot Control 2016:1–27.
[17] D'Amico JM, Condliffe EG, Martins KJ, Bennett DJ, Gorassini MA. Recovery of neuronal and network excitability after spinal cord injury and implications for spasticity. Front Integr Neurosci 2014;8:36.
[18] Floeter MK, Kohn AF. H-reflexes of different sizes exhibit differential sensitivity to low frequency depression. Electroencephalogr Clin Neurophysiol 1997;105:470–5.
[19] Trompetto C, Marinelli L, Mori L, Pelosin E, Curra A, Molfetta L, et al. Pathophysiology of spasticity: implications for neurorehabilitation. Biomed Res Int 2014;2014:354906.
[20] Sonner PM, Ladle DR. Early postnatal development of GABAergic presynaptic inhibition of Ia proprioceptive afferent connections in mouse spinal cord. J Neurophysiol 2013;109:2118–28.
[21] Misiaszek JE. The H-reflex as a tool in neurophysiology: its limitations and uses in understanding nervous system function. Muscle Nerve 2003;28:144–60.
[22] Danner SM, Krenn M, Hofstoetter US, Toth A, Mayr W, Minassian K. Body position influences which neural structures are recruited by lumbar transcutaneous spinal cord stimulation. PLoS One 2016;11:e0147479.
[23] Windhorst U. On the role of recurrent inhibitory feedback in motor control. Prog Neurobiol 1996;49:517–87.
[24] Barbeau H, Marchand-Pauvert V, Meunier S, Nicolas G, Pierrot-Deseilligny E. Posture-related changes in heteronymous recurrent inhibition from quadriceps to ankle muscles in humans. Exp Brain Res 2000;130:345–61.
[25] Calancie B, Broton JG, Klose KJ, Traad M, Difini J, Ayyar DR. Evidence that alterations in presynaptic inhibition contribute to segmental hypoexcitability and hyperexcitability after spinal-cord injury in man. Electroencephalogr Clin Neurophysiol 1993;89:177–86.
[26] Sarmadi A, Firoozabadi SM, Torkaman G, Fathollahi Y. Assessing information of soleous and gastrocnemius motor unit H-reflex response to paired stimulation. Electromyogr Clin Neurophysiol 2004;44:401–8.
[27] Wood SA, Gregory JE, Proske U. The influence of muscle spindle discharge on the human H reflex and the monosynaptic reflex in the cat. J Physiol 1996;497(Pt 1):279–90.
[28] Fedirchuk B, Nielsen J, Petersen N, Hultborn H. Pharmacologically evoked fictive motor patterns in the acutely spinalized marmoset monkey (Callithrix jacchus). Exp Brain Res 1998;122:351–61.
[29] Evarts EV. Motor cortex reflexes associated with learned movement. Science 1973;179:501–3.
[30] Porter R, Rack PM. Timing of the responses in the motor cortex of monkeys to an unexpected disturbance of finger position. Brain Res 1976;103:201–13.
[31] Wiesendanger M. Input from muscle and cutaneous nerves of the hand and forearm to neurones of the precentral gyrus of baboons and monkeys. J Physiol 1973;228:203–19.
[32] Chen R, Corwell B, Hallett M. Modulation of motor cortex excitability by median nerve and digit stimulation. Exp Brain Res 1999;129:77–86.
[33] Hirashima F, Yokota T. Influence of peripheral nerve stimulation on human motor cortical excitability in patients with ventrolateral thalamic lesion. Arch Neurol 1997;54:619–24.
[34] Komori T, Watson BV, Brown WF. Influence of peripheral afferents on cortical and spinal motoneuron excitability. Muscle Nerve 1992;15:48–51.
[35] Tokimura H, Di Lazzaro V, Tokimura Y, Oliviero A, Profice P, Insola A, et al. Short latency inhibition of human hand motor cortex by somatosensory input from the hand. J Physiol 2000;523 Pt 2:503–13.
[36] Chang YJ, Hsieh TH, Huang YM, Hsu MJ, Wong AMK. A lack of modulation of motor evoked potential in sensory-impaired individuals with spinal cord injuries. J Med Biol Eng 2011;31:37–43.
[37] Toth S, Solyom A, Vajda J. Frequency resonance investigation of the H Reflex. J Neurol Neurosurg Psychiatry 1979;42:351–6.
[38] Kagamihara Y, Hayashi A, Okuma Y, Nagaoka M, Nakajima Y, Tanaka R. Reassessment of H-reflex recovery curve using the double stimulation procedure. Muscle Nerve 1998;21:352–60.
[39] Zehr EP. Considerations for use of the Hoffmann reflex in exercise studies. Eur J Appl Physiol 2002;86:455–68.
[40] Deletis V, Schild JH, Beric A, Dimitrijevic MR. Facilitation of motor evoked-potentials by somatosensory afferent stimulation. Electroencephalogr Clin Neurophysiol 1992;85:302–10.
[41] Morita H, Shindo M, Yanagawa S, Yanagisawa N. Neuromuscular response in man to repetitive nerve-stimulation. Muscle Nerve 1993;16:648–54.
[42] Mizuno Y, Tanaka R, Yanagisawa N. Reciprocal group-1 inhibition on triceps surae motoneurons in man. J Neurophysiol 1971;34:1010.
[43] Nielsen J, Petersen N. Is presynaptic inhibition distributed to corticospinal fibers in man. J Physiol-London 1994;477:47–58.
[44] Thompson AK, Wolpaw JR. Targeted neuroplasticity for rehabilitation. Prog Brain Res 2015;218:157–72.