Characteristics of preceding Ia activity on postactivation depression in health and disease

Behdad Tahayori,1,2,3 Bahman Tahayori,4 and David Koceja1
1Department of Kinesiology and Program in Neurosciences, Indiana University, Bloomington, Indiana; 2Department of Physical Therapy, School of Rehabilitation Sciences, Shiraz University of Medical Sciences, Shiraz, Iran; 3Neurorehabilitation Program, The Crossing Rehabilitation Center, Indiana University Health, La Porte, Indiana; 4Neuroengineering Research Laboratory, Department of Electrical and Electronic Engineering, The University of Melbourne, Melbourne, Australia

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Tahayori B, Tahayori B, Koceja D. Characteristics of preceding Ia activity on postactivation depression in health and disease. J Neurophysiol 113: 3751–3758, 2015. First published April 22, 2015; doi:10.1152/jn.00132.2015.—Previous activation of the soleus Ia afferents causes a depression in the amplitude of the H-reflex. This mechanism is referred to as postactivation depression (PAD) and is suggested to be presynaptically mediated. With the use of a paired reflex depression paradigm (eliciting two H-reflexes with conditioning-test intervals from 80 ms to 300 ms), PAD was examined in a group of healthy individuals and a group of hemiplegic patients. Healthy individuals showed substantial depression of the test H-reflex at all intervals. Although the patient group showed substantially less depression at all intervals, increasing the interval between the two reflexes sharply reduced the depression. In a separate experiment, we varied the size of the conditioning H-reflex against a constant test H-reflex. In healthy individuals, by increasing the size of the conditioning H-reflex, the amplitude of the test H-reflex exponentially decreased. In the patient group, however, this pattern was dependent on the conditioning-test interval; increasing the size of the conditioning H-reflex caused an exponential decrease in the size of the test reflex at intervals shorter than 150 ms. This pattern was similar to that of healthy individuals. However, conducting the same protocol at a longer interval (300 ms) in these patients resulted in an abnormal pattern (instead of an exponential decrease in the size of the test reflex, exaggerated responses were observed). Fisher discriminant analysis suggested that these two patterns (which differed only in the timing between the two stimuli) were substantially different from each other. Therefore, it is suggested that the abnormal pattern of PAD in hemiplegic stroke patients could be a contributing factor for the pathophysiology of spasticity.

postactivation depression; H-reflex; stroke

DEPRESSION OF TRANSMISSION between Ia fibers and alpha motoneurons attributable to the history of activation of the fibers is a spinal inhibitory mechanism that occurs after a passive stretch of the agonist muscle, tapping its tendon or electrically stimulating the afferents repetitively or at a low frequency (Crone and Nielsen 1989). The term postactivation depression (PAD) or homosynaptic depression has been used to indicate that the same afferents are involved in this depression (Crone and Nielsen 1989). Modulation of this mechanism during functional tasks such as muscle activation and movement initiation (Stein et al. 2007) has been reported, but its functional role in shaping human movement is not very well understood (Knikou 2008).

In noninvasive experimental settings, this inhibitory mechanism can be quantitatively evaluated by different H-reflex protocols. One method is to elicit single pulses of nerve stimulation at different frequencies and measure the amplitude of the first and last H-reflex. Another method is to elicit two H-reflexes with equal stimulus intensities at a constant frequency but varying the interstimulus delay. The protocol is referred to as paired-reflex depression (PRD). In this protocol, the first H-reflex is regarded as the conditioning stimulus, and the second H-reflex is regarded as the test (conditioned) reflex. Studies over the past few decades have shed considerable light on the mechanism of this inhibition. It is widely agreed that the mechanism is confined to the presynaptic terminals of the Ia fibers and does not depend on alpha motoneuron excitability. Furthermore, PAD does not interfere with heteronymous sources to the motor pool (Beswick and Evanson 1957). Hultborn et al. (1996) demonstrated that this presynaptic inhibitory mechanism is not accompanied by GABAergic primary afferent depolarization and does not produce dorsal root potentials. They also concluded that this inhibition does not spread to the motoneuron pool and is strictly confined to those Ia fibers that have been previously activated.

PAD has also been examined in pathological conditions. It is known that this depression is substantially reduced in spastic patients and patients with multiple sclerosis and spinal cord injuries (Grey et al. 2008; Nielsen et al. 1995). Evidence also exists to suggest that the impairment in PAD contributes to the pathophysiology of spasticity and also correlates with hyperreflexia (Aymard et al. 2000). However, the exact mechanism that causes a reduction of PAD in pathology is not well understood. In these pathological conditions, the decrease of depression is more pronounced as the interval between the two stimuli increases (faster recovery of the test H-reflex). On the other hand, in pathological conditions, there is limited information regarding the effect of an unequal-sized conditioning H-reflex on the amplitude of the test H-reflex. In this study, we deviated from the traditional PRD method by varying the amplitude of the conditioning H-reflex against a constant test H-reflex. This new method enabled us to examine the interaction between the stimulus interval and the conditioning stimulation intensity and provided a new aspect of the pathophysiology of PAD.
Fig. 1. Experimental setup and data collection protocols. In the conventional method (A), 2 stimuli were delivered to the nerve at various intervals (inter-stimulus intervals, ISIs). Also, with the same setup and protocol, the stimulation intensity was increased to obtain the ratio of test/conditioning H-reflex at various percentages of maximum muscle response (M-max). In the alternative method (B), the 2 stimuli were delivered from 2 different constant current units and merged into a single pair of cables by a customized bridging cable. By changing the stimulation intensity of the first channel, we elicited different amplitudes of the conditioning H-reflex to a constant intensity test reflex.

METHODS

A total of 10 healthy controls (age = 26.4 ± 3.4 yr) and 10 hemiplegic stroke patients (age = 63.5 ± 12.0 yr) participated in this study. Healthy subjects self-reported having no neurological disorder. In this group, all measurements were performed on the right lower extremity. Subjects who had hemiplegic stroke had suffered from one cerebrovascular accident ranging from 2 mo to 5 yr before the time of data collection. In this group, all measurements were performed on the affected side. All subjects read and signed the consent form approved by the Institutional Review Board of Indiana University Bloomington and Indiana University Health La Porte Hospital.

A Therapeutic Unlimited EMG unit was used for electromyographic (EMG) data recording. A pair of Ag-AgCl surface electrodes with an internal diameter of 0.5 cm and a fixed inter-disc distance of 2 cm was placed above the Achilles tendon and over the soleus muscle, and another pair of electrodes was placed on the motor point of the tibialis anterior muscle and parallel to its fiber orientation. The ground electrode was placed on the lateral malleolus. The EMG signals were digitized at 4,000 Hz using a National Instrument A/D board (Austin, TX). Two constant current units (DS7A; Digitimer, Hertfordshire, UK) were used for nerve stimulation. A two-channel Grass stimulation unit was used to control the precise timing and the sequence of triggering of the two constant current stimulators.

The soleus H-reflex was elicited by placing a pair of stimulating bar electrodes with a fixed inter-disc distance of 2 cm over the posterior tibial nerve (PTN). Maximum muscle response (M-max) and maximum H-reflex (H-max) were measured. The amplitude of the H-reflex was set to be between 15 and 30% of M-max. The small M-waves on each trial were monitored to ensure consistent stimulation conditions. After each nerve stimulation (paired reflex or single pulse), at least a 10-s delay was given to allow full recovery of the H-reflex (Crone and Nielsen 1989; Knikou 2008).

PRD was used to assess PAD (Robertson and Koceja 2003) in all 10 healthy individuals and all hemiplegic patients. In this method, two stimuli were delivered to the PTN with intervals of 80, 150, 200, 250, and 300 ms between the conditioning and the test reflexes (Fig. 1A). At least five trials were elicited at each interval; when M-wave amplitude fluctuated >5%, the trial was repeated. The ratio of the test H-reflex to the conditioning H-reflex was measured at each tested interval to determine the amount of depression. For the statistical analysis of this part of the study, a two-way ANOVA (group × interval) was used (Keppel and Wickens 2004). Subsequently, one-sample t-tests were used to compare the test/conditioning H-reflex ratio to 1.0 to determine the presence of postactivation depression at the five selected intervals of 80, 100, 150, 200, and 300 ms (Cressie 1980).

To determine whether the ratio of the test/conditioning H-reflex in the conventional PRD method was affected by the amplitude of the reflexes, we performed the PRD method at different stimulation intensities. We used the same method illustrated in Fig. 1A but started from very low stimulation intensities and increased the intensity in a stepwise fashion until H-max was evoked. Any change in the inhibition ratio attributable to stimulation intensity would be a potential confounding factor for the second part of the study (explained in detail below). This preliminary part of the study was performed on 4 of the 10 healthy subjects and was tested at a fixed conditioning-test interval of 200 ms. For this experiment, the reflexes were normalized to M-max to assess whether the inhibition ratio was affected by the stimulation intensity and allowed us to use the correct stimulus intensity for the second part of this study.

In the second part of the study, we deviated from the conventional PRD method by delivering each pulse from one of the two stimulation units and thus had the ability to change the intensity of the first stimulus while maintaining the second at a constant intensity (Fig. 1B). In this protocol, we first set each stimulation unit separately to elicit equal-sized H-reflexes. After eliciting several single H-reflexes from each unit and ensuring that both units would produce the same
amplitude reflex, we then sequenced the two units to test the PRD at a fixed interval. After eliciting a few trials with the same intensities, we altered the stimulation intensity for the conditioning reflex, and at least five trials were elicited at that intensity. Subsequently, the amplitude was altered again, and a few trials were elicited until enough conditioning H-reflexes with amplitudes ranging from nearly 0 to 30% of M-max were elicited. In this alternative protocol, therefore, the test H-reflex was influenced by a variable conditioning H-reflex. The reflexes were then normalized to M-max and were plotted against each other.

This protocol was conducted on 6 of the 10 subjects in the healthy group. The interval between the conditioning and the test reflex was 300 ms in this experiment, and the amplitude of the control H-reflex was set to between 15 and 30% of the M-max. This protocol was also conducted in nine hemiplegic patients at two different intervals of 80 ms (a short interval) and 300 ms (a long interval). To classify these two groups of data points (short interval vs. long interval) quantitatively, we used Fisher linear-discriminant (FLD) analysis (Alpaydin 2004; Fisher 1936). FLD is one of the oldest, yet commonly used methods for discriminating data of different classes quantitatively. For two-dimensional data, the main idea is to find projections of samples from the known classes (2 classes in our problem) onto lines that are optimally separated. The objective function (J value) to be maximized is defined as the ratio of "between-class scatter" over "within-class scatter". The value of J quantitatively describes how well the known classes are separated. A detailed mathematical description of the FLD method is given in supplemental material; supplemental material for this article is available online at the Journal of Neurophysiology website.

RESULTS

PRD was tested in 10 healthy controls and 10 patients who had undergone stroke at 5 different conditioning-test intervals (as illustrated in Fig. 1A). Although both groups showed depression of the test reflex at short intervals, in the hemiplegic group, the depression showed almost full recovery at the 300-ms conditioning-test interval (Fig. 2). Statistical analysis showed a significant interaction between subject group and interval ($F_{3,54} = 3.90, P = 0.014$). There was a significant main effect of the intervals ($F_{3,54} = 28.12, P < 0.001$) and also for the subject group ($F_{1,18} = 30.53, P < 0.001$). Post hoc analysis with the α-level correction showed that the two groups were different at all conditioning-test intervals, revealing that hemiplegic patients had substantially less depression at each of the tested intervals. The existence of a statistical interaction between the two groups strongly suggests that the recovery of PAD is substantially different in these two groups. To infer at what interval the observed ratio was statistically equal to 1.0 (i.e., no substantial PAD), one-sample t-tests were performed. The results of this test in the hemiplegic patients showed that the ratio was significantly <1.0 at 80-ms conditioning-test interval ($t = 7.62, P < 0.0001$), was still depressed at 150 ms ($t = 2.66, P = 0.026$), but was not significant at the longer-latency intervals. Therefore, at short-latency intervals, PAD existed in these patients but to a lesser extent than the healthy group.

Fig. 3. Examining the relation between the conditioning H-reflex and the test H-reflex at different intensities. Using a single constant-current unit, we delivered 2 equal-size stimuli to the nerve. Therefore, the ratio was tested at different percentages of M-max. A: results of the responses elicited with the same intensity. In the case of no inhibition at any of the intensities, all the data points would fall on the identity line. Therefore, a shift to the left and up (above the identity line) represents depression of the test H-reflex. By increasing the intensity, we observed that, when the first H-reflex is between 15 and 30% of M-max, a linear relation exists in the amount of depression (delimited by the square). Inset: regression lines for the 4 subjects. All lines fall above the identity line. B: pooled data. The ratios were categorized based on the intensity of the conditioning H-reflex as a percentage of M-max. Keep in mind that the stimulation intensity for both reflexes was the same. It can be seen that the ratio is not being affected by the size of the conditioning H-reflex when the reflex is between 15 and 30% of M-max.
PRD was tested in four healthy subjects with a fixed conditioning-test interval of 200 ms at various stimulation intensities to test the effect of the size of the conditioning H-reflex on PAD (with the protocol illustrated in Fig. 1A). By increasing the stimulation intensity for eliciting paired reflexes, larger reflexes were elicited, and the ratio was calculated. It was observed that, by performing the PRD method with intensities that elicited conditioning H-reflexes between 15 and 30% of M-max, the ratios were constant. Figure 3A shows the data from a representative subject. The slope of this curve is the test/conditioning H-reflex ratio and was perfectly linear at the range delimited in Fig. 3. The inset is the fitted line for the four subjects. The mean $R^2$ for these four subjects was 0.84 ± 0.098. Figure 3B shows the pooled results of the ratio. Therefore, because, in the conventional PRD method, the ratio was constant when the conditioning H-reflex was between 15 and 30% of M-max, we selected this amplitude for the next experiment (explained below). Also, a nonlinear relation in the ratio (different sensitivities of the H-reflex to depression) would be a confounding factor, which was ruled out here.

To further investigate the pathophysiology of PAD, we used the alternative protocol at which an unequal-sized conditioning H-reflex was elicited against the test H-reflex (e.g., changing the amplitude of the conditioning H-reflex). In the healthy group, because the test H-reflex was substantially depressed at all intervals, we used a long interspike interval for this alternative protocol. This protocol was tested on six of the healthy subjects. Increasing the amplitude of the conditioning H-reflex produced a decrease in the size of the test reflex (Fig. 4A). Plotting the amplitudes of the test H-reflex against the variable conditioning H-reflex revealed a nonlinear pattern of change in the size of the test H-reflex as a result of increasing the amplitude of the conditioning H-reflex. These results are shown in Fig. 4B for all healthy subjects. The nonlinearity of the change between the two variables was determined by fitting the data points with a linear and an exponential model and measuring the goodness of fit (least squares). Fitting the data points obtained from this alternative method with a linear model yielded an $R^2 = 0.53 ± 0.09$, whereas a nonlinear exponent yielded $R^2 = 0.79 ± 0.1$. A statistical comparison between the linear $R^2$ ($LR^2$) and exponential $R^2$ ($eR^2$) showed that $eR^2$ significantly explained more of the variability of the data and was a better fit ($F_{1.5} = 37.41, P = 0.002$).

We conducted the same alternative protocol in nine hemiplegic stroke subjects. The protocol was conducted with two different conditioning-test intervals (as illustrated in Fig. 1B), a short interval at which maximum PAD had been observed and a longer interval at which no inhibition was observed. Figure 5 shows the data of one exemplar hemiplegic subject. As can be seen in this patient, the ratio was <1.0 at short intervals (Fig. 5A). Testing this ratio with different intensities at a short interval (in this subject at 100 ms) with the conventional PRD method (Fig. 1A) yielded a linear relation between the two. With most of the data points being above the identity line, the existence of PRD was indicated (Fig. 5B). Testing with the alternative method at this short interval also produced an exponential relation similar to that of healthy individuals (Fig. 5C). However, as the conditioning-test interval increased, the amount of inhibition of the test H-reflex decreased and eventually yielded a test reflex larger than the conditioning reflex (Fig. 5A, at intervals >250 ms). Testing the ratio at different intensities at a longer interval (in this patient at 300 ms) resulted in a linear relation with the data points being on or below the identity line (Fig. 5D), suggesting an exaggerated test H-reflex. Using the alternative method at this longer interval yielded a completely different relation (Fig. 5E). Comparing Fig. 5C with Fig. 5E suggests that conditioning the test H-reflex with a variable conditioning H-reflex yields different results depending on the timing between the two stimuli; at short intervals, it causes depression, whereas, at a long interspike interval, it can even cause facilitation. The only
The difference between these two conditions is the interval between the two reflexes.

To quantitatively measure this difference between the two conditions, FLD was used (details explained in METHODS and supplemental material). Figure 6A shows the results for both conditions in one hemiplegic patient, and Fig. 6B shows the J value of all nine hemiplegic subjects. A one-sample t-test showed that the J value (separation between the 2 conditions)
DISCUSSION

PAD was examined in healthy and hemiplegic subjects by using PRD at various conditioning-test intervals. We observed that, in the hemiplegic patients, PAD was generally reduced and also had a much faster recovery; i.e., the depression completely disappeared at the conditioning-test interval 300 ms. In healthy individuals, a full recovery takes up to 10 s (Crone and Nielsen 1989). The characteristics of PAD were further examined by using an alternative protocol in which the amplitude of the conditioning reflex was different from that of the test reflex. In healthy individuals, increasing the size of the conditioning reflex exponentially decreased that of the test reflex. In the patient group, however, the changes in the test reflex were dependent on the conditioning-test interval; we observed that performing the same protocol at short intervals produced a pattern similar to that of healthy subjects. However, performing exactly the same protocol at a longer interval produced a pathological response in these patients.

As is shown in previous studies that have used the H-reflex method for conditioning protocols such as recurrent inhibition, reciprocal inhibition, and cutaneous inhibition (Crone et al. 1990), our data also showed that using an H-reflex with an amplitude between 15 and 30% of M-max is a suitable size for this study because the ratio of test/conditioning H-reflex was fairly constant in this range. As was expected, in the healthy group, the ratio of test/conditioning H-reflex was significantly <1.0 up to 300 ms. However, it is known that the depression of the test H-reflex can last up to several seconds after the conditioning stimulation (Clair et al. 2011; Crone and Nielsen 1989; Robertson and Kocaja 2003; Robertson et al. 2012). With our new method of using a conditioning H-reflex of variable size for the PRD method, a nonlinear pattern emerged in the healthy group; increasing the size of the conditioning H-reflex exponentially decreased that of the test H-reflex. One possible explanation for this behavior is that, by increasing the stimulation intensity of the conditioning reflex, the synapses of larger-sized motoneurons are being activated and therefore in the subsequent stimulation are more likely to be depressed. Another possibility is that the exponential change in the amplitude of the test H-reflex is partly related to the type of muscle fibers and the size of the motor unit being affected. Also, the strength of stimulation may affect the number of motoneurons at the sublimal fringe (Pierrot-Deseilligny and Mazevet 2000). However, with a higher intensity, these motoneurons are being recruited. As such, there is a possibility that some of the Ia fibers, which also make synapses with these motoneurons, are likewise subject to depression and therefore exponentially affect the size of the test reflex. A final possibility is that the activation of Ia fibers affects other Ia fibers, which were not involved in the first stimulation but were activated by the second stimulation. None of these possibilities are mutually exclusive. Although it was suggested in the 1960s that PAD activates long loop reflexes (Taborikova and Sax 1969), later intracellular experiments suggested that this type of inhibition does not spread to other Ia fibers (Hultborn et al. 1996). Regardless of the physiological reason for this phenomenon, we established normative data to compare with the pathological condition.

In the hemiplegic group, the linear relation of test/conditioning H-reflex at various intensities (conventional method, Fig. 1A) also held with the difference that, at longer intervals, the ratio was close to or even above 1.0 (Fig. 5, B and D). Reduced PAD has been reported previously in pathological and spastic conditions (Grey et al. 2008; Nielsen et al. 1993, 1995). This impairment is also suggested to be correlated with spasticity and motor impairment (Burke et al. 2013). However, it is not well understood how this depression is related to the exaggeration of the stretch reflex.

Initial experiments on presynaptic inhibition with primary afferent depolarization suggested that this mechanism is reduced in hemiplegic spastic conditions. Subsequently, it was believed that the increase in neurotransmitter release is due to the impaired presynaptic inhibition. However, later studies have shown that presynaptic inhibition is intact or minimally impaired in upper motoneuron lesions (Aymard et al. 2000; Burke and Ashby 1972; Faist et al. 1994; Katz 1999; Lamy et
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Nielsen et al. (2009) suggested that stretch reflex hyperexcitability is likely due to mechanisms other than presynaptic inhibition. These mechanisms may contribute to changes in the efficacy of transmission on the Ia-motoneuron synapse. Anatomical changes and neuronal organization such as establishment of new connections might also contribute to the exaggeration of reflexes (Dietz and Sinkjaer 2007). This study provided new insight into the pathophysioloogy of the exaggerated reflex in hemiplegic patients. Our results suggest that the interval between the conditioning and the test reflexes plays a critical role in the pathology. We observed that increasing the amplitude of the conditioning reflex caused an exaggerated test reflex mainly at long intervals, whereas, at short intervals, the pattern was similar to that observed in healthy individuals. This finding and the discrepancy between the effect of a short and a long interval on the test H-reflex cannot be readily explained by an increase of neurotransmitter release. Under normal physiological conditions, as time elapses, more glutamate transmitters are restored by high-affinity sodium-dependent glutamate transporters (Danbolt 2001). More importantly, it has been shown in the cat that baclofen reduces transmitter release in the Ia-α motoneuron synapses (Jimenez et al. 1991), but its administration in spastic patients with multiple sclerosis did not increase the reduced PAD (Ørsnes et al. 2000). To the best of our knowledge, no study has been conducted to determine whether the reuptake mechanism is impaired in hemiplegic stroke. Therefore, with our current understanding, it is likely that mechanisms other than the increase in neurotransmitter release also contribute in the time-dependent exaggeration of the test H-reflex. Considering the complex collateral arborization of Ia fibers with the dendrites of α-motoneurons (Brown and Fyffe 1978, 1981; Lev-Tov et al. 1983), it is likely that the activation of Ia fibers invades other Ia synapses in normal physiological conditions, and this invasion of action potentials might be reduced in pathological conditions. However, with our current data, this assumption remains at the hypothetical level.

Modern theories of the pathophysiology of spasticity and hyperreflexia suggest that part of the issue is the alteration in muscle fibers and properties secondary to the central nervous system damage (Dietz and Sinkjaer 2007). There is a possibility, therefore, that the observed exaggerated reflex response could be attributable to muscle potentiation at long intervals where the muscle properties have enough time to cause potentiation (Xenofondos et al. 2014). However, with the current experimental design, we are unable to differentiate between spinal pathologies and muscular pathologies. More importantly, the current study investigated the reflex pathway passively. Although we observed a clear time-dependent impairment in PRD, it cannot be inferred how and to what extent this impairment contributes to movement disorders associated with hemiplegia.

We concluded that the results of this study showed a more complex aspect of the pathophysiology of PAD in hemiplegia than originally observed. It was demonstrated that the time interval between the conditioning and the test reflex plays a critical role in producing a normal or a pathological response in patients who have undergone stroke. Our alternative protocol that consisted of using a variable conditioning H-reflex provides new insight into the pathophysiology of PAD in hemiplegia; in the absence of any other changes in the conditioning protocol, only changing the interval between the two stimuli alters a normal pattern to an abnormal pattern in hemiplegic patients who have undergone stroke. Therefore, it appears that the timing of Ia fiber activation has a critical role in the pathophysiology of hemiplegic spasticity. This finding could be due to an impairment in the spread of PAD in the motoneuron pool. This alternative approach might prove beneficial in examining the effectiveness of post-stroke intervention protocols that target spinal cord pathways.

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Disclosures

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

Author Contributions

Author contributions: Behdad Tahayori and D.K. conception and design of research; Behdad Tahayori and D.K. performed experiments; Behdad Tahayori and Bahman Tahayori analyzed data; Behdad Tahayori, Bahman Tahayori, and D.K. interpreted results of experiments; Behdad Tahayori and Bahman Tahayori prepared figures; Behdad Tahayori and Bahman Tahayori drafted manuscript; Behdad Tahayori and D.K. edited and revised manuscript; Behdad Tahayori, Bahman Tahayori, and D.K. approved final version of manuscript.

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