



## Invited review

## The H-reflex as a probe: Pathways and pitfalls

Maria Knikou<sup>a,b,c,\*</sup><sup>a</sup> Health Sciences Doctoral Programs, City University of New York, Staten Island, NY 10314, USA<sup>b</sup> Department of Physical Medicine and Rehabilitation, Feinberg Medical School, Northwestern University, Chicago, IL 60611, USA<sup>c</sup> Sensory Motor Performance Program, Rehabilitation Institute of Chicago, Chicago, IL 60611, USA

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## ABSTRACT

The Hoffmann (or H) reflex is considered a major probe for non-invasive study of sensorimotor integration and plasticity of the central nervous system in humans. The first section of this paper reviews the neurophysiological properties of the H-reflex, which if ignored create serious pitfalls in human experimental studies. The second section reviews the spinal inhibitory circuits and neuronal pathways that can be indirectly assessed in humans using the H-reflex. The most confounding factor is that reciprocal, presynaptic, and Ib inhibition do not act in isolation during movement. Therefore, characterization of these spinal circuits should be more comprehensive, especially in cases of a neurological injury because neurophysiological findings are critical for the development of successful rehabilitation protocols. To conclude, the H-reflex is a highly sensitive reflex with an amplitude that is the result of complex neural mechanisms that act synchronously. If these limitations are recognized and addressed, the H-reflex constitutes one of the major probes to assess excitability of interneuronal circuits at rest and during movement in humans.

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## 1. Introduction

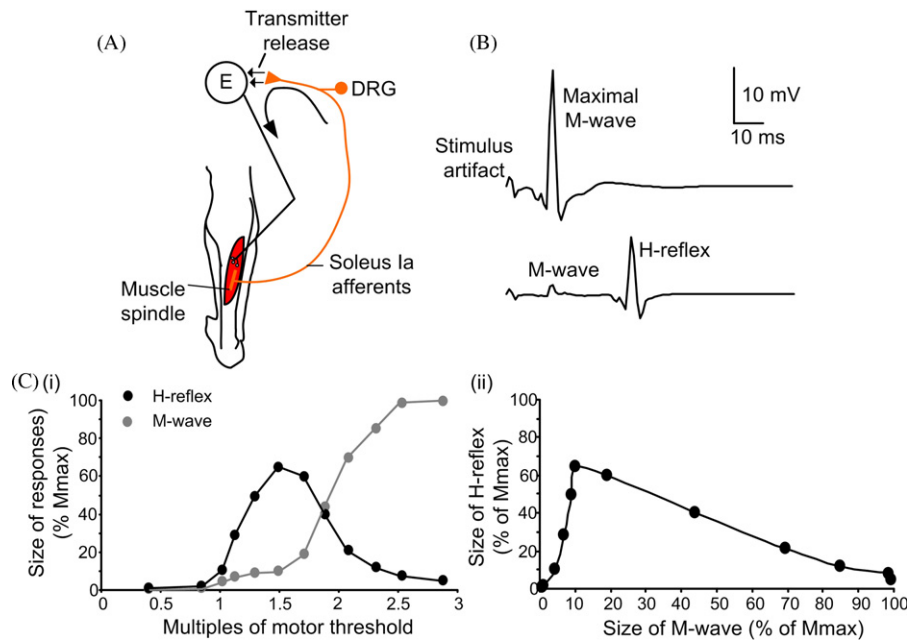
The Hoffmann (or H) reflex is one of the most studied reflexes in humans and is the electrical analogue of the monosynaptic

stretch reflex. The H-reflex is evoked by low-intensity electrical stimulation of the afferent nerve, rather than a mechanical stretch of the muscle spindle, that results in monosynaptic excitation of  $\alpha$ -motoneurons (Fig. 1A). Hence, the H-reflex bypasses the muscle spindle and the fusimotor activity that may influence the sensitivity of the Ia afferents to engage a 'simple' reflex circuit.

The purpose of this brief review is to discuss specific neurophysiological characteristics of the H-reflex and the methods available

\* Correspondence address: Health Sciences Doctoral Programs, The Graduate Center, City University of New York, 2800 Victory Boulevard, Building 5N-207, Staten Island, NY 10314, USA. Tel.: +1 718 982 3316; fax: +1 718 982 2984.

E-mail addresses: [knikou@mail.csi.cuny.edu](mailto:knikou@mail.csi.cuny.edu), [m-knikou@northwestern.edu](mailto:m-knikou@northwestern.edu).



**Fig. 1.** The “simple” H-reflex pathway. Stimulation of the posterior tibial nerve at the popliteal fossa below motor threshold results in excitation of Ia afferents that largely induce monosynaptic excitation of homonymous  $\alpha$ -motoneurons (A), which is evidenced on the EMG as an H-reflex. At maximal stimulus intensities the maximal M-wave (B) is observed without an H-reflex being present (C). The soleus H-reflex/current and M-wave/current recruitment curves constructed in one subject while standing are indicated in the left panel. In the right panel, the H-reflex/M-wave recruitment curve is indicated for the same recordings shown in the left panel. In both graphs, the H-reflex and M-wave are presented as a percentage of the  $M_{\max}$ . The reflex recruitment curve shown is not representative because differences across subjects are usually observed (From Knikou M, unpublished observations).

to study spinal inhibitory circuits and neuronal pathways in man while using the H-reflex. H-reflexes can be elicited in nearly all muscles where access to their mixed peripheral nerve is possible. However, this review is focused on the soleus H-reflex because its amplitude and spinal inhibitory mechanisms have been extensively used to assess the neural basis of movement and spasticity in humans with and without neurological injury.

## 2. Neurophysiological characteristics of the H-reflex

Electrical stimulation of a mixed peripheral nerve above motor threshold (MT) produces two responses in the homonymous muscle, an M-wave (short-latency direct motor response due to stimulation of motor axons) and an H-reflex (Fig. 1B). At supra-maximal stimulation, the H-reflex is absent due to collision of the antidromic motor volley with the orthodromic afferent volley and the M-wave is maximum ( $M_{\max}$ ).

The H-reflex and M-wave do not recruit the same  $\alpha$ -motoneurons. Alpha motoneurons are recruited in an orderly fashion from smallest (more excitable with large Ia excitatory postsynaptic potentials; EPSP) to largest (less excitable smaller Ia EPSP) (Henneman et al., 1965). Thus, small motoneurons innervating slow motor units are recruited first in the H-reflex, while electrical stimulation that elicits the M-wave activates larger diameter axons that innervate fast motor units.

The amplitudes of the M-wave and H-reflex vary significantly with the level of stimulus intensity. In Fig. 1C(i) the M-wave and H-reflex are plotted as a function of the stimulus strength (expressed in multiples of MT), while in Fig. 1C(ii) the H-reflex is plotted against the corresponding M-wave. The slope of the ascending limb of the H-reflex/stimulus recruitment curve warrants attention because it provides information about the reflex gain (input–output relationship) (Mazzocchio et al., 2001; Pastor and Valls-Sole, 1998). Further, this slope is best fitted with a sigmoid (and not a linear) function that takes into consideration the amplitude of the maximal H-reflex

( $H_{\max}$ ) and the stimulus required to evoke a response equivalent to half the  $H_{\max}$  (see more details in Klimstra and Zehr, 2008). The reflex gain can also be estimated as the slope between motoneuron activation (measured as background EMG activity) and H-reflex amplitude (Larsen and Voigt, 2004). It is essential to determine this parameter in studies conducted during movement, because changes in slope may be related to changes in the number of the  $\alpha$ -motoneurons that are considered to be at a subliminal fringe<sup>1</sup> (Capaday and Stein, 1987), since stimuli activate motoneurons in proportion to the level of motor activity and the size of the associated subliminal fringe (Devanne et al., 1997).

One of the most confounding assumptions in human reflex studies is that the H-reflex derives from group Ia afferents that project monosynaptically to  $\alpha$ -motoneurons. Oligosynaptic inputs have ample time to contribute to the H-reflex given that the composite EPSPs of the H-reflex in the soleus motoneurons have a sufficiently long rising phase to permit oligosynaptic inputs to reach the  $\alpha$ -motoneurons (Burke et al., 1984). Further, when a test H-reflex is elicited at the descending portion of the recruitment curve (see Fig. 1C), the slow motoneurons that are mostly engaged in this reflex are insensitive to facilitation or inhibition (Crone et al., 1990), and it is highly possible for it to be influenced by Ib and recurrent inhibitory pathways (Pierrot-Deseilligny et al., 1981).

To avoid these confounding factors, it is my personal experience that the control H-reflex should be evoked only on the ascending portion of the recruitment curve at 20–40% of the  $M_{\max}$  across subjects. Concomitantly, the M-wave amplitude should remain stable and be approximately 4–8% of the  $M_{\max}$ , because a stable M-wave suggests that a constant number of motor nerve fibers and thus Ia afferents, are excited by the test stimuli (Crone et al., 1990;

<sup>1</sup> An electrical stimulus causes a few motoneurons to fire but it induces sub-threshold excitation to many other motoneurons that constitute the subliminal fringe (Pierrot-Deseilligny and Mazevet, 2000).

Boorman et al., 1996). However, it is possible for the H-reflex to reach an amplitude of 50–60% of the  $M_{\max}$  when the M-wave is absent. Nonetheless, the M-wave amplitude during an experiment is an important point of reference and should be monitored so its size is similar under all tested conditions. This will ensure that the observed reflex modulation pattern (when present) is not due to changes in the composition of the test afferent volley but to mechanisms that act to depress or facilitate the H-reflex (see next section).

It is widely held that the H-reflex measures motoneuron pool excitability. Based on this assumption, it was suggested that the ratio of  $H_{\max}/M_{\max}$  estimates motoneuron pool excitability (Funase et al., 1994). However, since the magnitude of the  $H_{\max}$  depends on pre- and post-synaptic events (see next section on spinal inhibitory mechanisms), and the amount of concomitant antidromic activity elicited in the motor nerve axons (Misiąszek, 2003), it is certain that this ratio cannot estimate motoneuron pool excitability.

The state of excitability of the  $\alpha$ -motoneuron pool plays a significant role in determining the H-reflex magnitude. In order to maintain stable motoneuron excitability and minimize post-synaptic effects, H-reflex recordings should be conducted during voluntary, sustained, homonymous muscle contractions and not at rest (see more details about muscle contraction and H-reflex in Stein and Thompson, 2006). More specifically, H-reflex recordings are conducted at 5–10% (or higher) of the maximal voluntary contraction (MVC) of the homonymous muscle. If the H-reflex is recorded during voluntary sustained contraction of the homonymous muscle, the amplitude of the H-reflex may be affected by changes of  $\alpha$ -motoneuron excitability due to descending excitation, contraction-associated sensory feedback, decrease of Ib inhibition (Marchand-Pauvert et al., 2002), and changes in recurrent inhibition or even presynaptic inhibition (Hultborn et al., 1987a).

When reflex recordings are conducted with subjects at rest, influences from supraspinal centers are anticipated to be minimal and spinal inhibitory interneurons such as Renshaw cells and Ia–Ib inhibitory interneurons, that are affected by muscle contraction, might be less active. However, there is no way to assess the excitability state of the cells or their sub-threshold excitability level, which may vary within and across subjects. This is the most significant drawback when reflex recordings are conducted while the homonymous muscle is in a relaxed state.

A major issue that needs attention is that during various motor tasks, low threshold motoneurons (the ones that are mostly associated with human H-reflex studies) not involved in the muscle contraction might be equally excited (Pierrot-Deseilligny, 1997). To counteract this phenomenon, several studies have proposed that the reflex gain should be similar across tasks (Capaday and Stein, 1987; Larsen and Voigt, 2004). However, the gain should also be estimated for muscles not involved in the motor task, because the reflex gain of the antagonistic muscles differs during concentric and eccentric contraction of the agonist muscle (Sekiguchi et al., 2003). The input/output properties of the H-reflex are based on the number of sensory fibers that are synchronously excited to make motoneurons discharge (DeBruin et al., 2006). However, when a movement is attempted a marked shift in the relationship of Ia input/motoneuron output has been observed (DeBruin et al., 2006). The H-reflex might have a different recruitment gain for various motor tasks even in the same subject (Kernell and Hultborn, 1990).

Taken altogether, we may assume that motoneuron excitability and actions of spinal interneurons are minimal when reflex recordings are conducted during different tasks at similar low-muscle contraction levels. However, it should be noted that sensory afferent input might combine differently during different motor tasks.

### 3. The H-reflex as a probe to study spinal neuronal pathways and mechanisms

Changes in H-reflex amplitude following a conditioning stimulus are usually employed to assess post-synaptic events or changes in the amount of the presynaptic inhibition acting on Ia afferent terminals. This is because the amplitude of the test reflex depends on the motoneuron excitability and the ongoing presynaptic inhibition of Ia fibres that mediate the test afferent volley. Thus, the H-reflex can be used as a probe to study spinal neuronal pathways and mechanisms at rest and during movement in humans.

#### 3.1. Monosynaptic Ia excitation and homosynaptic depression

The amplitude of the H-reflex depends on the history of previously activated Ia afferents, even when variables that influence the H-reflex amplitude are kept constant. This phenomenon is known as homosynaptic or post-activation depression and occurs at the level of the synapse between the soleus Ia afferents and  $\alpha$ -motoneurons (see Fig. 1A).

In cats, it has been shown that depressed subsequent motoneuron Ia EPSPs elicited at low frequency (e.g. 3/s) are associated with a decrease in the amount of neurotransmitter released (Kuno, 1964). The depression of the EPSP, driven mostly by the previously activated Ia afferents in humans, has been attributed to similar mechanisms as those described in cats (Crone and Nielsen, 1989a; Hultborn et al., 1996). This depression is likely localized at the presynaptic terminal (Crone and Nielsen, 1989a; Kohn et al., 1997; Voigt and Sinkjaer, 1998) although not related to the classic GABAergic presynaptic inhibition, which is discussed in the next section.

Homosynaptic depression is reported to be of functional significance in the neonatal rat spinal cord (Lev-Tov and Pincus, 1992) and depends on the size of the H-reflex with respect to the  $M_{\max}$  (Floeter and Kohn, 1997). It is reduced during voluntary contraction of the homonymous muscle and is abolished in standing human subjects when the soleus muscle is contracting at 15–20% of MVC (Stein et al., 2007). This depression is dramatic when consecutive H-reflexes are elicited at short inter-stimulus intervals of 1–2 s and decreases progressively as the interval increases. However, an inter-stimulus interval as long as 10 s is required for this reflex depression to vanish completely (Aymard et al., 2000; Crone and Nielsen, 1989a). Thus, the longer the inter-stimulus interval the smaller the homosynaptic depression.

Post-activation H-reflex depression may arise from passive stretch or voluntary contraction of the tested muscle, and Ia afferent discharges elicited by the stretch of the tested muscle during contraction of its antagonist (Crone and Nielsen, 1989a; Hultborn et al., 1996). In order to eliminate the effects of movement-mediated post-activation depression, test and conditioning stimuli have to be triggered at the very onset of movement or at least 8 s after the end of the preceding movement (Crone and Nielsen, 1989a).

Reduced homosynaptic depression may underlie muscle stiffness and spasticity in people with neurological injury at the spinal or supraspinal level (Aymard et al., 2000; Grey et al., 2008), or in rats with mid-thoracic spinal contusions (Thompson et al., 1998). Unfortunately, the functional role of homosynaptic depression in humans is poorly understood.

#### 3.2. Presynaptic inhibition of Ia afferents

Afferent input flows constantly to the spinal cord from various sources including skin, muscles, tendons, and joints. This sensory feedback needs to be controlled (through inhibition or dis-facilitation) for a motor task to be executed. The point

where sensory feedback from the periphery might be effectively controlled is at the presynaptic inhibitory synapses of afferent terminals on  $\alpha$ -motoneurons.

Frank and Fuortes (1957) described depression in the size of the EPSP without any detectable changes in the resting membrane potential or the excitability of postsynaptic cells in the cat. This presynaptic inhibition was accompanied by primary afferent depolarization and caused by axo-axonal gamma-aminobutyric (GABA) synapses that reduced the size of the presynaptic impulse that led to decreased liberation of excitatory transmitters and consequently decreased the monosynaptic transmission of the Ia excitatory effects (Rudomin and Schmidt, 1999). Presynaptic inhibition and primary afferent depolarization are generally considered to be mediated by the same interneurons. These interneurons are activated by group I afferents, inhibited by flexor reflex afferents, and controlled by descending tracts (Jankowska, 1992).

Presynaptic inhibition can arise from a number of sources and constitutes an inhibitory mechanism associated with modulation of monosynaptic reflexes under numerous conditions. Changes in the amount of presynaptic inhibition acting on Ia afferent terminals has been associated with soleus H-reflex modulation during ipsilateral or bilateral passive leg movements in humans (Brooke et al., 1993; Nikou, 2006; Nikou and Rymer, 2002), passive ankle dorsiflexion (Morita et al., 2001), and standing (Katz et al., 1988). Further, presynaptic inhibition has been suggested to account partly for the differences observed in the soleus H-reflex amplitude at equivalent EMG levels during walking, standing, and running (Capaday and Stein, 1987; Morin et al., 1982). This finding supports the notion that presynaptic inhibition is capable of changing the reflex amplitude during a motor task regardless the excitation level of the  $\alpha$ -motoneurons. It is worth noting that conclusions regarding modulation and functional significance of the ankle stretch reflex cannot be made on evidence derived from H-reflex studies because the soleus H-reflex is more sensitive to presynaptic inhibition than the mechanically evoked ankle stretch reflex (Andersen and Sinkjaer, 1999; Morita et al., 1998).

At the onset of voluntary contraction in the human lower limb, the amount of presynaptic inhibition acting on Ia afferent terminals in the contracting muscle is decreased (Hultborn et al., 1987b; Iles and Roberts, 1987), probably due to descending control (Meunier and Pierrot-Deseilligny, 1998; Nielsen and Kagamihara, 1993). It is widely accepted that different subsets of interneurons trans-

mit presynaptic inhibition to Ia terminals projecting to various motoneuron pools. Thus at the onset of voluntary contraction, there is a differential control of presynaptic inhibition of Ia afferent terminals projecting to motoneurons of the contracting muscle and to other motor nuclei (Hultborn et al., 1987b), particularly the antagonist motoneuron pools (Meunier and Morin, 1989). This differential control could be due to cortical control since transcranial stimulation increases heteronymous Ia facilitation and decreases D1 inhibition, while the decrease in presynaptic inhibition of Ia afferents to motoneurons of the contracting muscle persists after an ischaemic blockage of group I afferents from the contracting muscle (Meunier and Pierrot-Deseilligny, 1998).

To conclude, presynaptic inhibition is critical to neural control of movement since it gates sensory afferent feedback to the spinal cord to assist in smooth execution of a movement or motor task. Adjustment of presynaptic inhibition of Ia afferents to motoneurons that are (or not) involved in contraction may contribute to the production of muscle synergy or movement patterns suited to the motor task performed.

3.2.1. Protocol for studying presynaptic inhibition in humans

Different methods have been proposed for assessing presynaptic inhibition in humans, including comparison between changes in EMG activity and H-reflex amplitude (Schieppati and Crenna, 1984), and soleus H-reflex depression following tibialis anterior, biceps femoris or Achilles tendon vibration (Burke et al., 1976; Hultborn et al., 1987a; Morin et al., 1984).

In this section, details will be provided for protocols that use the soleus H-reflex as a test reflex. A conditioning afferent volley to the common peroneal (CP) (train of 3–5 shocks, 1–1.4 $\times$  MT) or radial nerve (single shock, 0.7–1 $\times$  MT) evokes several phases of H-reflex depression in the antagonist muscles, e.g. soleus and flexor carpi radialis (FCR) (Berardelli et al., 1987; Crone et al., 1987). The early soleus H-reflex depression involves the reciprocal Ia inhibitory pathway and is exerted at a postsynaptic level, which is discussed in the next section. The soleus H-reflex depression that appears at conditioning–test (C–T) intervals that range from 6 to 30 ms is called D1 inhibition, and is believed to be mediated by presynaptic inhibition of soleus Ia afferents (Crone and Nielsen, 1994; Pierrot-Deseilligny, 1997) (Fig. 2A). In Fig. 2B, soleus H-reflex depression by CP nerve stimulation at a MT level is indicated for C–T intervals of 60–120 ms. However, CP nerve stimulation results in a long lasting

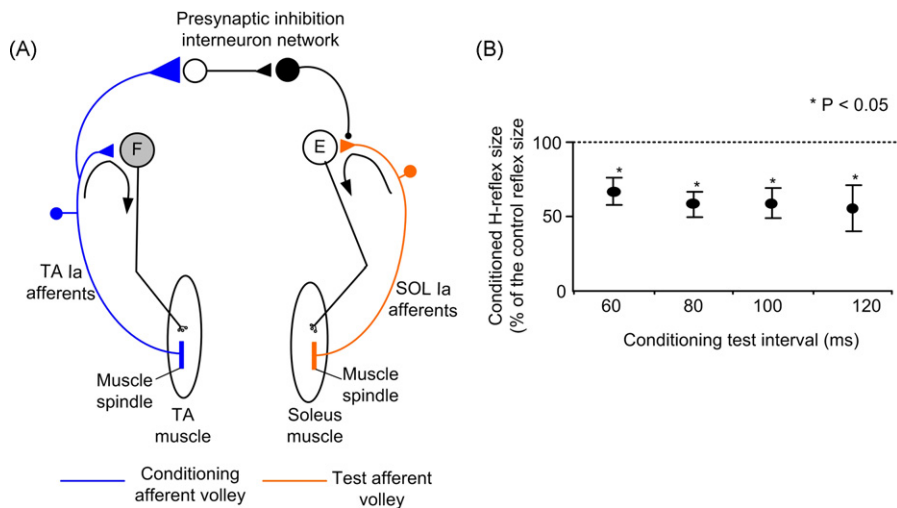


Fig. 2. Presynaptic inhibition of Ia afferents induced by a conditioning afferent volley. Common peroneal (CP) nerve stimulation at low intensities is delivered before posterior tibial nerve stimulation to establish based on the amplitude of the conditioned soleus H-reflex the amount of presynaptic inhibition acting on soleus Ia afferent terminals (A) and average size of the soleus H-reflex conditioned by CP nerve stimulation at C–T intervals ranged from 60 to 120 ms for 10 seated subjects (B) (data adopted and modified from Nikou and Taglianetti, 2006).

(up to 200 ms) soleus H-reflex depression, similar to that observed in the cat (Eccles, 1964; Eccles et al., 1962).

In humans, the same conditioning afferent volleys do not modify cortical-evoked responses in the soleus or FCR muscles (Berardelli et al., 1987; Faist et al., 1996), which supports the presynaptic nature of the soleus and FCR H-reflex depression. In this context, D1 soleus H-reflex depression may reflect an increased excitability of the primary afferent depolarization interneurons that might be driven by strong afferent inputs generated by movement (Capaday et al., 1995). Under this scenario, the circuits involved in the presynaptic inhibition might become saturated and stop responding to the conditioning afferent volley, as reported during human walking (Faist et al., 1996).

A method to study presynaptic inhibition of Ia afferents in humans was proposed and developed by Hultborn et al. (1987a). This method involves the assessment of heteronymous Ia facilitation exerted by quadriceps afferents onto soleus motoneurons, and relies on the heteronymous monosynaptic projections from the quadriceps to soleus motoneurons. This method was demonstrated by motor unit studies and verified in animals by direct intracellular recordings (Fournier et al., 1986; Hultborn et al., 1987a).

The femoral nerve (conditioning stimulus) is stimulated with a single pulse (1-ms duration) through a monopolar ball electrode placed at the femoral triangle, while the indifferent electrode is placed on the gluteus maximus muscle. The stability of the conditioning stimulation strength is verified by a stable M-wave in the vastus lateralis muscle. Femoral nerve stimulation is delivered after posterior tibial nerve because the former is closer to the spinal cord and thus the C–T intervals are negative by convention. The involved neuronal pathway and the time course of the heteronymous soleus H-reflex facilitation are shown in Fig. 3.

During the first 0.5 ms of femoral nerve induced soleus H-reflex facilitation, the monosynaptic Ia excitation is not contaminated by any other events and the soleus H-reflex facilitation depends on the size of the conditioning Ia monosynaptic EPSP (Fournier et al., 1986; Hultborn et al., 1987a). Thus, the changes observed on the conditioned soleus H-reflex amplitude during the first half ms that the soleus H-reflex facilitation is established reflect modifications of the on-going presynaptic inhibition of heteronymous Ia afferent terminals. Simply stated, the larger the conditioned soleus H-reflex facilitation the smaller the on-going presynaptic inhibition. Under

the same principles, the on-going presynaptic inhibition can be indirectly assessed by examining the amount of soleus H-reflex facilitation by inferior soleus nerve stimulation in the first half ms (Pierrot-Deseilligny et al., 1981).

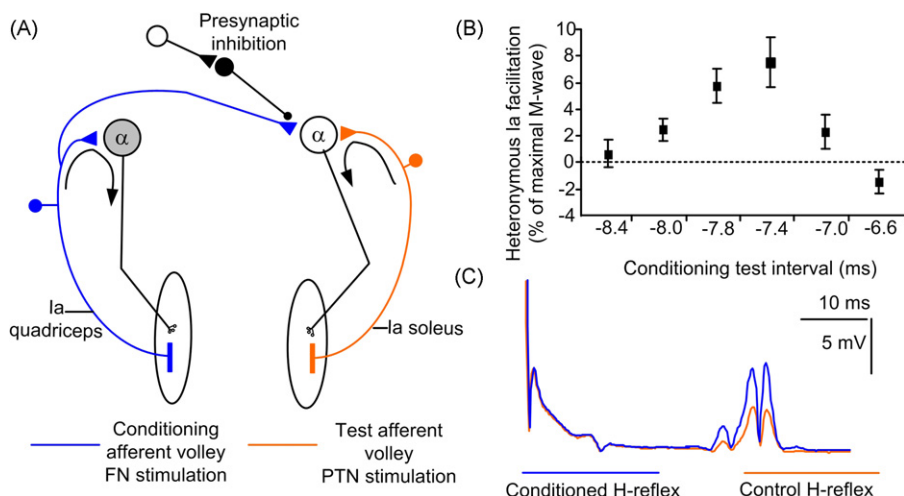
At this point it should be made clear that heteronymous monosynaptic Ia facilitation and D1 inhibition differ significantly. The former tests the on-going presynaptic inhibition exerted on Ia afferents involved in the conditioning afferent volley, and the latter method tests the effects of activation of the presynaptic network by a conditioning (electrical or mechanical) afferent volley.

Regardless of the method used, the above assumptions can be made only when the recruitment gain of the  $\alpha$ -motoneurons remains stable, especially if the test is conducted during voluntary muscle contraction. To confidently demonstrate changes in on-going presynaptic inhibition, similar findings should be observed in motor unit studies using the post-stimulus time histogram method (Katz et al., 1988; Nielsen and Kagamihara, 1993). When this is not possible, a change in the recruitment gain can be eliminated when the amount of monosynaptic facilitation and vibration-induced reflex depression occur in the opposite direction.

Further, the amplitude of the conditioned H-reflex should be similar to that of the unconditioned (or test) reflex, since its sensitivity to facilitation and inhibition depends on the size of the control H-reflex (Crone et al., 1990). Therefore, when the conditioning stimulus is delivered and the reflex size increases or decreases the intensity should be adjusted appropriately. However, this adjustment may affect the recruitment reflex gain (input–output relation of the test reflex) (Kernell and Hultborn, 1990), which in turn may influence low- and high-threshold motoneurons differently. Thus, to ensure that the effects are not due to changes in the reflex recruitment gain, the H-reflex should be accompanied by a stable M-wave or more direct methods, such as motor unit studies.

### 3.3. Reciprocal Ia inhibition

Ia afferents participate in neuronal pathways that inhibit the antagonist  $\alpha$  motoneurons subserving a reciprocal activation pattern between the agonist and corresponding antagonist muscles during movement in humans. The existence of this neural pathway was first postulated in humans by Hoffmann who showed that the soleus H-reflex is decreased when the pretibial (antagonistic)



**Fig. 3.** Presynaptic inhibition of Ia afferents reflected by changes of heteronymous Ia facilitation. (A) Sketch illustrates the spinal circuit during which femoral nerve (FN) stimulation at low intensities delivered after posterior tibial nerve stimulation induces monosynaptic excitation of soleus  $\alpha$ -motoneurons. Changes in the amount of heteronymous Ia facilitation reflect modulation of the on-going presynaptic inhibition acting on the Ia afferents of the conditioning afferent volley (quadriceps Ia afferents), (B) time course of soleus H-reflex facilitation by FN stimulation in one seated subject and (C) full-wave waveform rectified averages ( $n=20$ ) of the control and conditioned H-reflex following FN stimulation at  $-7.8$  ms are shown. Note that the heteronymous Ia reflex facilitation occurred without a significant change in the size of the M-wave (data adopted and modified from Knikou, 2006).

muscles are contracting (Hoffmann, 1952). Through intracellular recordings this neuronal pathway was later described in detail (Eccles et al., 1956), and is now known that this pathway probably involves one interneuron, referred as Ia inhibitory interneuron. Ia inhibitory interneurons are excited by corticospinal, rubrospinal, and vestibulospinal tracts (Grillner and Hongo, 1972; Hongo et al., 1969) as well as by flexion reflex and group II, III and IV muscle afferents (Jankowska, 1992). Reciprocal Ia inhibition exerted from ankle flexors onto plantar flexors can be seen 50 ms before the onset of tibialis anterior (TA) muscle contraction (Crone et al., 1987), further supporting the supraspinal initiation of this spinal inhibition in humans.

### 3.3.1. Protocol for studying reciprocal Ia inhibition in humans

One method for studying short-latency reciprocal inhibition involves measuring the H-reflex amplitude following stimulation of the antagonist peripheral nerve. In the human lower limb, reciprocal inhibition is mostly studied between ankle flexors and extensors given its significant role in soleus H-reflex depression during the swing phase of walking in humans (Ethier et al., 2003). However, studies have attributed the H-reflex depression during the swing phase of gait to supraspinal centers (Schneider et al., 2000).

The CP nerve is stimulated with a single pulse (1-ms duration) through a bipolar electrode placed distal to the head of the fibula at C–T intervals of 2–4 ms to determine the reciprocal inhibition exerted by ankle flexors on extensors. TA activity must be present without peroneal muscle activity because peroneal muscles are not antagonists to the soleus muscle (Meunier et al., 1993), thus their activity might obscure any reciprocal inhibition. The involved neuronal pathway and an example of soleus H-reflex depression by CP nerve stimulation at a C–T interval of 2 ms are shown in Fig. 4.

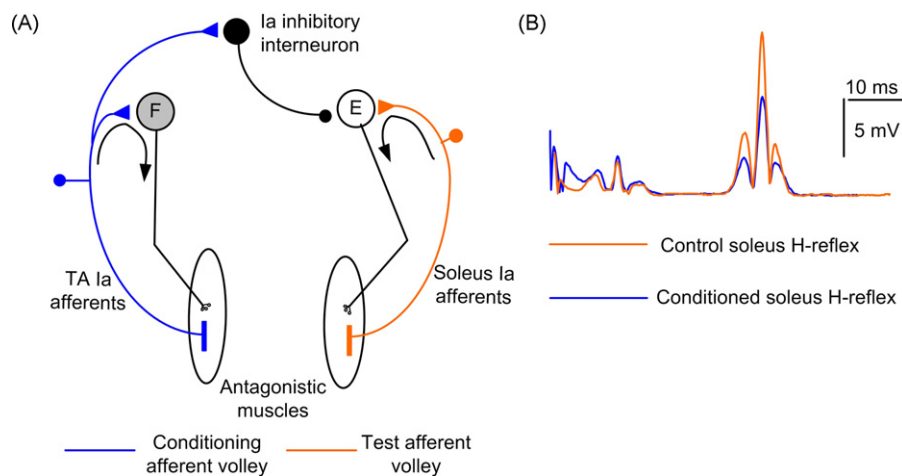
Reciprocal inhibition can be elicited at conditioning stimulus intensities below, at, or above MT (Crone and Nielsen, 1989b; Crone et al., 1985, 1987; Kido et al., 2004a). A conditioning electrical stimulus delivered below MT is likely to excite only TA Ia afferents. However, the absence of a TA M-wave would make nearly impossible to quantify the constancy of the conditioning stimulus during the experiment. The TA H-reflex could potentially be used as an indicator of conditioning stimulus constancy. However, the TA H-reflex is rarely observed in healthy subjects at rest (Crone et al., 1987 and personal unpublished observations), probably because sustained voluntary TA activation is required for observing the TA H-reflex (Baret et al., 2003). If reciprocal inhibition is studied

during TA muscle contraction in order to evoke a TA H-reflex that can be used as a reference, other problems can arise since reciprocal inhibition is stronger at the onset of ankle dorsi flexion (Crone and Nielsen, 1989b; Morita et al., 2001) and the strength of Ia inhibitory interneuronal actions differ when subjects are at rest or moving voluntarily.

If the conditioning stimulus is delivered above MT ( $1.1–1.5 \times$  MT) there is a possibility that reciprocal Ia inhibition will be contaminated by Ib afferent discharges (Pierrot-Deseilligny et al., 1981). Further, the conditioning stimulus (CP nerve) may excite group II and cutaneous afferents as well as motor efferents. Excitation of efferents may lead to activation of Renshaw cells, which would affect the amount of reciprocal inhibition through recurrent inhibition (Baret et al., 2003; Hultborn et al., 1971; Katz et al., 1991). The soleus H-reflex depression might be stronger at  $1.5 \times$  the TA MT, but it cannot be attributed solely to TA Ia afferents since other neuronal pathways might also be involved.

Reciprocal inhibition depends greatly on the size of the test soleus H-reflex (Crone et al., 1985). Maximal reciprocal inhibition can be observed when the control soleus H-reflex ranges from 5 to 15% of the  $M_{max}$ . Lastly, the subjects participating in a study should be of similar age, and subject group populations should be age-matched when comparing the amount of reciprocal inhibition in patients and healthy subjects (Kido et al., 2004b).

The method described to study reciprocal inhibition in humans is indirect so actions from other inhibitory interneurons at a post- or presynaptic level cannot be fully distinguished. In cats, Ia afferent terminals exert presynaptic inhibition on Ia inhibitory interneurons, while interneurons that mediate presynaptic inhibition are likely to project to the synapses of Ia afferents on the Ia inhibitory interneurons (Eccles et al., 1963; Enriquez-Denton et al., 2000). This means that depression of the antagonists might be a combination of reciprocal and presynaptic inhibition (and possibly recurrent inhibition), probably tuned by the brain (Lavoie et al., 1997). Thus in human studies, especially during movement, it is difficult to distinguish the contribution of each of these mechanisms on the observed reflex depression. To counteract this limitation during movement, it has been suggested that the conditioning effects of CP nerve stimulation should be observed on the soleus EMG activity instead of the soleus H-reflex (see more details in Stein and Thompson, 2006). Under this scenario, however, we should keep in mind that the amount of reciprocal inhibition depends on the type (dynamic vs. isometric) of contrac-



**Fig. 4.** Reciprocal Ia inhibition. (A) Spinal circuit designates the pathway of reciprocal inhibition exerted from ankle flexors following common peroneal (CP) nerve stimulation onto the soleus H-reflex. Reciprocal inhibition involves the Ia inhibitory interneuron and is exerted at a postsynaptic level and (B) waveform averages of 20 control and conditioned (by CP nerve stimulation) soleus H-reflexes evoked every 5 s at a conditioning test interval of 2 ms are illustrated for a healthy subject while seated at rest. Conditioning stimulus intensity was delivered at the tibialis anterior motor threshold level (data adopted and modified from Knikou and Taglianetti, 2006).

tion in the antagonistic muscles during movement (Crone et al., 1987).

#### 3.4. Non-reciprocal (or Ib) inhibition

Golgi tendon organs are force-sensitive receptors that respond to active and passive muscle force found at the muscle-tendinous junction situated in series with the muscle fibers. Afferents (Ib) from Golgi tendon organs are mostly excited by muscle load and participate in neuronal pathways that inhibit motoneurons projecting to synergists and facilitate motoneurons projecting to antagonists. The inhibition projecting to synergists was first described by Granit (1950) and referred as autogenetic inhibition. Actions of Ib afferents were tested by observing the conditioning effects of graded electrical stimulation of various hindlimb muscle nerves on monosynaptic test reflexes. Because these effects were opposite to the myotatic reflex pattern, they were referred to as the inverse myotatic reflex (Laporte and Lloyd, 1952). With intracellular recordings from motoneurons and electrical stimulation of muscle afferents (based on the threshold differences of Ia and Ib afferents) the pattern of inverse myotatic reflex was confirmed in the low spinal cat (Eccles et al., 1957a,b), but excitatory and inhibitory effects were found to be more widely distributed than those observed with monosynaptic reflexes. An example was that information from Ib afferents of an individual muscle could reach nearly all motor nuclei of the limb (Hongo et al., 1969).

The Ib inhibition was regarded as a protective mechanism against muscle overloading due to the high threshold of the Golgi tendon organs to passive stretch. However, this view was soon abandoned when it was demonstrated that active contraction of only a few or even a single motor unit could activate Golgi tendon organs (Houk and Henneman, 1967). The functional role of autogenetic inhibition was proposed by Houk and colleagues (Houk, 1979; Houk and Rymer, 1981), who suggested that this neuronal mechanism is important for regulating muscle stiffness. The stiffness hypothesis suggests that it is not the muscle length that is regulated by reflex actions but rather the muscle stiffness. The central idea was that the nervous system must be provided with information about the force generated by a muscle. This information is provided by the combined actions of the Golgi tendon organs and stretch reflex, so to maintain constant muscle stiffness during movement (Rothwell, 1987).

The excitatory and inhibitory Ib reflex actions on ipsilateral  $\alpha$ -motoneurons are relayed through di- or tri-synaptic linkages (Eccles et al., 1957a; Laporte and Lloyd, 1952), in which Ia afferents (Fetz et al., 1979), cutaneous, and joint afferents are involved (Eccles et al., 1957a,b). In humans at rest, stimulation of low threshold cutaneous afferents from the foot depress Ib inhibitory pathways to quadriceps motoneurons (Pierrot-Deseilligny et al., 1981), and reverse to excitation during voluntary muscle contraction. The latter was postulated due to a decrease of Ib inhibition from the medialis gastrocnemius (MG) nerve on the quadriceps H-reflex (but not on the soleus motoneurons) during voluntary contraction of the triceps surae (Pierrot-Deseilligny et al., 1982). These effects are likely to involve a presynaptic component since presynaptic inhibition of Ib terminals has been clearly documented (Lafleur et al., 1992). Postsynaptic inhibition produced by group I volleys has a GABAergic component mediated by interneurons that share pathways with those producing presynaptic inhibition (Rudomin et al., 1990).

The most important role of load-sensitive receptors in the ankle extensors is probably their contribution to the reflex regulation of locomotion and more specifically their critical contribution to the timing of different phases in the locomotor cycle. Conway and colleagues demonstrated in acute spinal cats (fictive locomotion was initiated by intravenous injection of nialamide or L-DOPA) that a

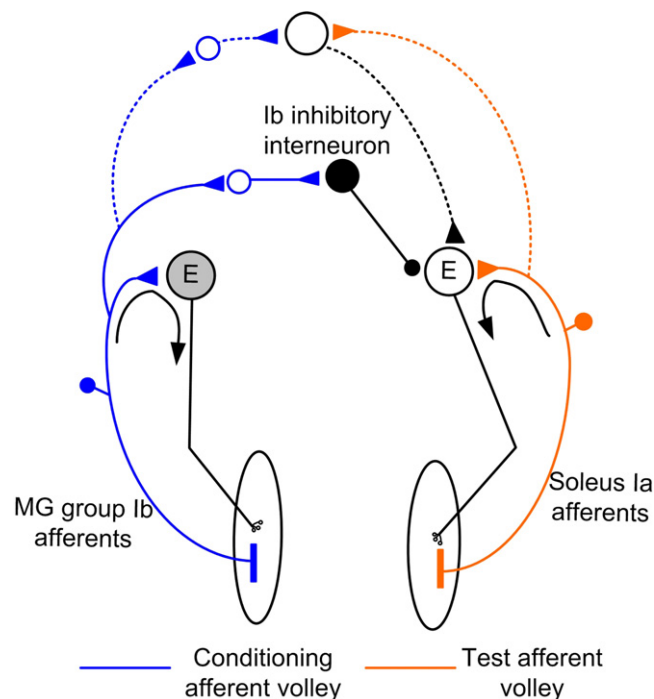
short train of volleys in the extensor group I afferents could reset the locomotor rhythm by stopping the flexion phase and promoting a new extension phase (Conway et al., 1987). These effects were verified to be mediated by Ib afferents (Conway et al., 1987), but recent studies have shown that group I extension enhancement during locomotion is also evoked by Ia afferents (Guertin et al., 1995). It has become pertinent that during stepping, activity of group I ankle extensor afferents reinforces the ongoing extensor activity and prevents the initiation of flexor activity (Conway et al., 1987; Duysens and Pearson, 1980; Pearson and Collins, 1993). Intracellular recordings support the idea that interneurons in the locomotor related Ib pathways are a part of the spinal rhythm generator for locomotion in mammals (Gossard et al., 1994; see reviews of Pearson, 1995 and Pearson et al., 1998).

The abolishment of the short-latency Ib inhibition and the opening of a new facilitatory group I pathway during walking have also been demonstrated in humans (Fig. 5) (Stephens and Yang, 1996). More recently, it was shown that Ib inhibition decreases during loading, and reverses to Ib facilitation during walking in humans (Faist et al., 2006). Ib inhibition is enhanced in decerebrate animals when compared to normal animals (Whelan and Pearson, 1997), suggesting that spinal animals may rely more on this positive sensory feedback.

To conclude, load-sensitive receptors in the ankle extensors are an integral part of reflex regulation of walking, reinforcing weight bearing during the stance phase of gait.

##### 3.4.1. Protocol for studying Ib inhibition in humans

The Ib afferents of the extensor muscles and specifically those of the ankle are considered gravitational load receptors that contribute to upright human posture. Thus, studies examining the Ib effects have mostly concentrated on the ankle plantar flexors. The method developed to non-invasively study short-latency Ib inhibition in humans involves measuring the soleus H-reflex



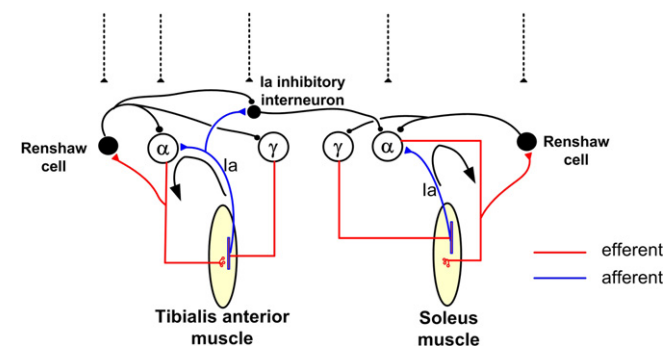
**Fig. 5.** Non-reciprocal group I inhibition. Spinal circuit designates the neuronal pathway engaged on the soleus H-reflex depression following medialis gastrocnemius (MG) nerve stimulation at group I threshold. Dotted lines denote a neuronal network that is manifested only during the stance phase of locomotion in humans and animals, whereas Ib inhibition from MG to soleus reverses to excitation.

following stimulation of the synergist MG nerve at a C–T interval of 6 ms. Because the MG nerve in non-human primates contains more Ib than Ia afferents (Hongo et al., 1984), it was proposed that MG nerve actions on the soleus H-reflex are mediated by Ib inhibition consistent with a disynaptic pathway (Pierrot-Deseilligny et al., 1979, 1981).

The conditioning stimulation electrode is placed 7–10 cm distal and medial to the cathode electrode for the posterior tibial nerve where a clear contraction of the MG muscle can be seen. The stimulus anode for the MG nerve is placed over the anterolateral portion of the leg just distal to the patella. Ib inhibition is obtained at conditioning stimulus intensities below MT to ensure that the effects observed are not contaminated by recurrent inhibition (Rossi et al., 1994). Thus, the MG MT needs to be checked throughout the experiment to ensure that the stimulus intensity is sub-threshold, and that the MG MT level does not change during the experiment. The conditioning stimulus to the MG nerve can be either a single (1 ms) or multiple pulses. Multiple conditioning pulses are more effective in generating the short-latency soleus H-reflex depression in humans (Bouaziz et al., 1975; Pierrot-Deseilligny et al., 1979).

### 3.5. Recurrent inhibition

Inhibitory neurons participate in neuronal circuits that subserve movement. One of the first identified inhibitory neurons was the Renshaw cell located in the ventral horn medial to the motor nuclei (Renshaw, 1946). Renshaw cells are excited by axon collaterals from motoneurons and provide recurrent inhibition of  $\alpha$ -motoneurons that project to the same or synergistic muscles. Activity in segmental afferents may influence Renshaw cells apart from the indirect excitation that is produced by motoneuronal reflex discharge. Polysynaptic excitation has been described after stimulation of dorsal roots, ipsilateral group II and III muscle afferents, cutaneous afferents, and contralateral flexor reflex afferents (see references in Baldissera et al., 1981), while Renshaw cells receive inhibition from ipsilateral and contralateral segmental afferents. In addition to their well known projection to  $\alpha$ -motoneurons, Renshaw cells connect with  $\gamma$ -motoneurons, the interneurons mediating reciprocal Ia inhibition, other Renshaw cells, and receive inputs from both primary afferents and descending tracts (Hultborn et al., 1971; Mazzocchio et al., 1994; see extensive review of Baret et al., 2003; Katz and Pierrot-Deseilligny, 1998) (Fig. 6). The wide convergence from a number of segmental reflex pathways suggests that the local feedback regulation provided by recurrent inhibition is not stereotyped and hard-wired but versatile in nature.



**Fig. 6.** Recurrent inhibition. Spinal circuit denotes the neuronal pathway of Renshaw cells and their connections to  $\alpha$ - and  $\gamma$ -motoneurons, and Ia inhibitory interneurons between ankle flexors and extensors. Renshaw cells depress the activity of  $\alpha$ - $\gamma$  motoneurons, and Ia inhibitory interneurons. Broken lines indicate parallel control of  $\alpha$ -motoneurons, Ia inhibitory interneurons, and Renshaw cells by the brain; closed circles: inhibition, closed triangles: facilitation.

Recurrent inhibition has mostly been described in terms of a stabilizing or limiting feedback mechanism that reduces the sensitivity of neurons to changes in their excitatory drive and decreases the frequency of their discharge to a given input when compared to a system without recurrent feedback. However, recurrent inhibition restricts motoneuron discharge by inhibiting motoneurons in the subliminal fringe (Brooks and Wilson, 1959), stabilizes the discharge frequency from tonically firing motoneurons (Granit et al., 1960), inhibits motoneurons to slow contracting muscle fibers during rapid contractions (Eccles et al., 1961), synchronizes motoneuron discharge patterns (Mattei et al., 2003), and increases short-term synchronization of  $\alpha$ -motoneuron discharges (Uchiyama and Windhorst, 2007).

Further, since there is Renshaw facilitation (increasing recurrent inhibition) during a weak tonic voluntary contraction, but Renshaw cell inhibition (suppressing recurrent inhibition) during a strong contraction, it has been suggested that recurrent inhibition may operate as a gain regulator of motor output (Hultborn and Pierrot-Deseilligny, 1979b). For example, when Renshaw cells are facilitated during a weak muscle contraction (Hultborn and Pierrot-Deseilligny, 1979a,b) the slope of the input–output relation is reduced thus providing a mechanism to control motor output. In contrast, Renshaw cell inhibition during strong contractions ensures a high input–output gain for the motoneuron pool favoring a large muscle force output. In addition, recurrent inhibition is reduced during soleus muscle contraction but is enhanced during voluntary contraction of the TA muscle when the subject is standing without support (Pierrot-Deseilligny et al., 1977), and when reciprocal inhibition of the antagonists is required (Mazzocchio et al., 1994). These findings strongly suggest that recurrent inhibition is engaged in motor tasks when equilibrium is threatened, and plays a role during selection of “appropriate” muscle synergy patterns.

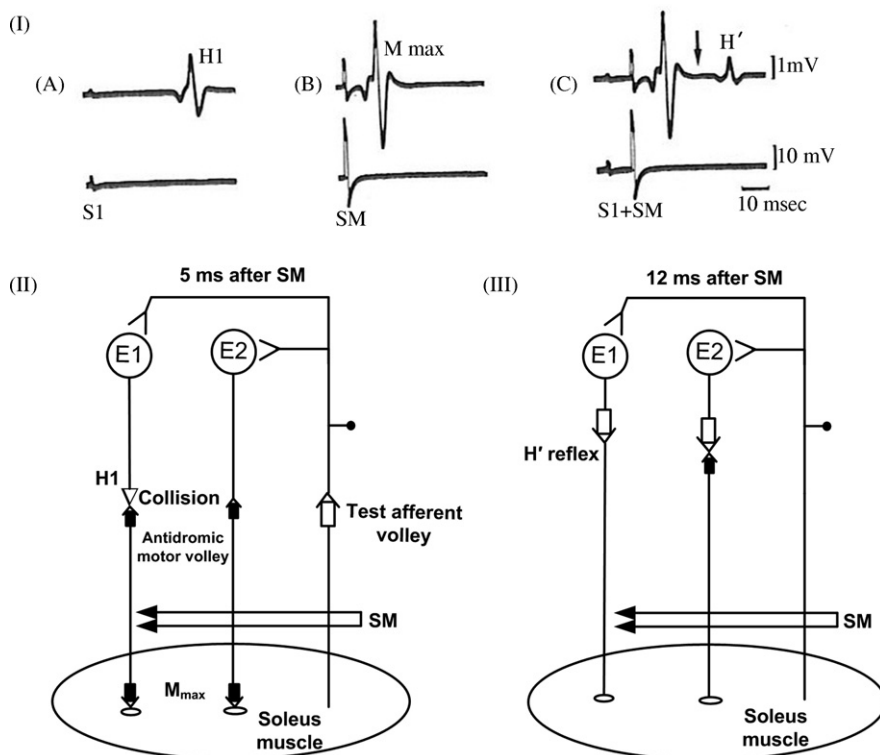
Renshaw cell activity during rhythmic motor tasks such as locomotion has been studied in the cat using extracellular recordings (Pratt and Jordan, 1987) and during locomotor-like activity in spinal cord preparations *in vitro* (Nishimaru et al., 2006). These studies have shown that Renshaw cells are rhythmically active and that their firing properties are modulated by motoneurons as well as by ipsilateral and contralateral locomotor networks. To conclude, it is apparent that recurrent inhibition plays a significant role in the neural control of movement.

#### 3.5.1. Protocol for studying recurrent inhibition in humans

Heteronymous recurrent inhibition in humans can be assessed by establishing the effects of femoral nerve stimulation on the soleus H-reflex or EMG activity. As previously described, femoral nerve stimulation at group I level induces facilitation of the soleus H-reflex at negative C–T intervals. The soleus H-reflex facilitation however is followed by a depression that is believed to be mediated by recurrent inhibition because it increases with the conditioning H-reflex, it has a short central delay, and lasts up to 40 ms (Bussel and Pierrot-Deseilligny, 1977). By analogy, heteronymous recurrent inhibition can be demonstrated using the quadriceps H-reflex preceded by a conditioning stimulus delivered to the inferior soleus nerve at a C–T interval of 22 ms (Iles et al., 2000).

The method for demonstrating homonymous recurrent inhibition was proposed by Pierrot-Deseilligny and colleagues (Bussel and Pierrot-Deseilligny, 1977; Pierrot-Deseilligny and Bussel, 1975) and relies on activating Renshaw cells with a conditioning soleus H-reflex discharge. More specifically, due to collision in motor axons between the orthodromic conditioning discharge and the antidromic motor volley from the strong test stimulus, the excitability is assessed only for motoneurons that have already fired in the first conditioning discharge.





**Fig. 7.** Homonymous recurrent inhibition in humans. (I) A stimulus S1 delivered to the posterior tibial nerve elicits an H1 reflex in the soleus muscle (A), while a supramaximal (SM) stimulus induces a maximal direct motor response ( $M_{max}$ ) (B) without an H-reflex to be present on the EMG because the antidromic motor volley collides with and eliminates the H-reflex evoked by the SM stimulus. When the stimulus S1 is delivered at an interval of 10 ms before the SM stimulus, the H1 is no longer present but a new response called H' (C) appears in the EMG. The diagrams II and III illustrate the different impulses, identified as arrows, propagating along the nerve fibres at C–T intervals of 5 and 12 ms. The Ia afferent volley induced by the SM test stimulus activates two motoneurons (E1 and E2). The white small arrow in diagram II represents the H1 reflex discharge in axon E1. White large arrows represent the Ia afferent test volley due to the test stimulus (II) and the following reflex discharge (III). Black arrows indicate the orthodromic motor volley evoking  $M_{max}$  and the antidromic motor volley due to stimulation of motor axons by the SM test stimulus (II). Five milliseconds after the SM test stimulus, impulses travel both orthodromically in Ia fibres and antidromically in motor axons. The H1 response, which runs along the E1 axon collides with and eliminates the antidromic motor volley. Twelve milliseconds after the SM test stimulus, a reflex response develops in both motoneurons E1 and E2. This response is blocked in motoneuron E2 but not in motoneuron E1 because the antidromic impulse in motoneuron E1 was erased by the H1 response, as shown in diagram II. The diagram I was borrowed from Pierrot-Deseilligny et al. (1976), with permission, and diagrams II and III were adopted and modified from Hultborn and Pierrot-Deseilligny (1979a,b).

A stimulus S1 delivered to the posterior tibial nerve elicits an H1 reflex while a supramaximal (SM) stimulus induces a maximum direct response ( $M_{max}$ ) (Fig. 7I) not followed by an H-reflex because the antidromic motor volley collides with and eliminates the H-reflex evoked by the SM stimulus (see motoneuron E2 in Fig. 7III). When the stimulus S1 is delivered at a sufficient C–T interval (10–35 ms) before the SM stimulus, the H1 reflex (due to afferent conditioning volley) collides with the antidromic motor volley caused by the test stimulus (see motoneuron E1 in Fig. 7III). This collision eliminates the antidromic volley and thus the H' test reflex due to the SM test stimulus can pass along the motor axons at the same latency as that of H1 (Pierrot-Deseilligny et al., 1976). The collision of the H1 with the antidromic motor volley following the SM test stimulus opens the way for an orthodromic reflex to the same SM test stimulus (Hultborn and Pierrot-Deseilligny, 1979a). The difference of  $H1 - H'$  reflects the amount of recurrent inhibition, while contribution of Ib inhibition is minimal given that its duration is less than 10 ms in humans (Pierrot-Deseilligny et al., 1981). The conditioning H-reflex should be stable and be 10% of the  $M_{max}$  without an M-wave present in order to eliminate the possibility that the stimulus activates other inhibitory afferent fibers.

The relation between H1 and H' reflex and the progressive increase in H' reflex depression depends on the after-hyperpolarization (AHP) of the motoneurons that discharge after the conditioning volley preventing the H' amplitude

from growing further. Further, the H' reflex depression depends on the growing inhibitory activity of Renshaw cells orthodromically activated by the conditioning H1 reflexes (Mazzocchio and Rossi, 1989). In order to attribute the effects solely to recurrent inhibition from that of AHP of  $\alpha$ -motoneurons, Mazzocchio and Rossi (1989) measured the recurrent inhibition following administration of a cholinergic substance (L-acetylcarnitine; L-Ac) since Renshaw cell excitation comes largely from motor axon collaterals mediated by the action of acetylcholine. Results indicated that L-Ac administration did affect the amplitude of the H', but most importantly that the amount of recurrent inhibition was dependent on the size of the conditioning H-reflex (H1), as originally proposed by Pierrot-Deseilligny et al. (1976).

Recurrent inhibition can be measured with subjects either at rest or during muscle contraction. As previously noted, the H' reflex is supramaximal for motor axons. When such a stimulus is delivered alone during muscle contraction a voluntary ( $V_1$ ) response, which is an electrophysiological variant of the H-reflex, can be seen after the  $M_{max}$  on the EMG (Hultborn and Pierrot-Deseilligny, 1979a). During voluntary contraction, the antidromic motor volley due to a SM stimulus would be abolished in some of motor axons by collision with the natural orthodromic impulses generated by the voluntary effort (see Fig. 2 in Aagaard et al., 2002). As a result of this collision, only a limited reflex volley is allowed to pass which is recorded as a V-wave (Hultborn and Pierrot-Deseilligny, 1979a; Upton et al., 1971). To avoid overestimation of the H' response and thus recur-

rent inhibition, the  $V_1$  responses should be subtracted from the corresponding H' reflexes.

#### 4. Clinical implications

The H-reflex can be utilized to assess modulation of spinal inhibitory interneuronal circuits, but attention is needed to the factors previously discussed that affect Ia transmission. The H-reflex is not hard-wired but is dramatically modulated during various motor tasks (task dependence) or during different phases of a cyclical movement (e.g. cycling and walking), and can be affected by several factors that must be acknowledged to avoid misinterpretation of the data. This is especially important when these mechanisms are assessed in people with a neurological injury.

All of the spinal inhibitory circuits described above (homosynaptic depression, reciprocal Ia inhibition, presynaptic inhibition, Ib inhibition, and recurrent inhibition) have been correlated to some degree with spasticity (for references see Nielsen et al., 2007), especially when the lesion occurs at the spinal cord. Presynaptic and postsynaptic inhibition might be differentially organized at cervical and lumbar levels so a characterization of these circuits is necessary to understand the organization of these circuits following spinal cord lesions at different segmental levels. Further, given the numerous factors that affect spinal reflex excitability, neurophysiological differences driven by the type or extent of the lesion, and spinal excitability state across patients, it is clear that a more systematic characterization of these interneuronal circuits should be conducted.

This is extremely important in light of new evidence. For example, homosynaptic depression can be increased after just a single cycling session in spinal-intact subjects (Meunier et al., 2007), and after 10 treadmill training sessions in one SCI subject capable of ambulation (Trimble et al., 1998), suggesting that the H-reflex can be used as a tool to study short- and long-term plasticity of the nervous system. This is further supported by the elegant studies conducted by Wolpaw and colleagues (for references see Wolpaw, 2007) whereas up- or down H-reflex conditioning training resulted in changes of motoneuron properties. In this line, conditioning H-reflex protocols that lead to locomotor recovery in rats (Chen et al., 2006) may be developed for people with neurological injuries in the near future.

#### 5. Conclusions

The H-reflex has been utilized as a probe to study neuronal pathways and spinal inhibitory control systems that are tightly coupled with the neural control of movement in health and neurological disorders. However, the reflex magnitude can change dramatically during contraction or stretch of agonist and antagonist muscles. Given the differences in motoneuron excitability state across subjects, differential supraspinal control of spinal inhibitory interneurons, and our inability to distinguish the relative contribution of each spinal inhibitory mechanism to motoneuronal excitability during a motor task or condition, it is clear that great attention should be paid to all of the limiting factors discussed in this review and be taken into consideration when data are interpreted. If these limitations are recognized and addressed, the H-reflex will remain one of the major probes for studying sensorimotor integration and training-induced neural adaptation in health and neural pathology.

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