The Journal of Physiology

PERSPECTIVES

A motor physiology recurrent topic: simplify assumptions to gain extra insight

Francisco J. Alvarez
Department of Physiology, Emory University, Atlanta, GA, 30322, USA
Email: francisco.j.alvarez@emory.edu

Edited by: Janet Taylor & Richard Carson

For those in need of a humbling experience with regard to the functional complexity of even the simplest synaptic circuits in the mammalian CNS, they should look no further than the first discovered inhibitory circuit in the spinal cord. The role of Renshaw cell-mediated recurrent inhibition (RI) of motoneurons continues to be contentious despite the fact that the basic circuitry was discovered over 70 years ago (Renshaw, 1946). RI is frequently described as a simple feedback circuit formed between Renshaw cells and the motoneurons they receive excitatory input from (homonymous RI); however, it is actually more complex. Renshaw cells receive further excitatory and inhibitory inputs from other sources (segmental and descending) and affect motoneuron activity in a task- and context-specific manner. Renshaw cells also distribute inhibition to motor pools different from those they receive inputs from (heteronymous RI) and thus are hypothesized to help select specific temporal and spatial combinations of motor units and pools during different motor acts. Importantly, Renshaw cells also target spinal circuits controlling reciprocal inhibition of antagonist muscles during flexion-extension movements and are believed to stabilize individual joints by modulating the co-contraction of antagonists. Despite the accumulation of hypotheses, RI function on motor control is frequently difficult to discern and demonstrate. Furthermore, Renshaw cell dysfunction is suspected in many diseases, including spasticity due to stroke or spinal cord injury, amyotrophic lateral sclerosis and other neurological diseases associated with gait and motor problems. Analyses of RI in disease can give insights into normal RI function in addition to helping understand pathophysiology, but testing the integrity of RI in patients is open to varied interpretations and results are sometimes hotly debated. This is the consequence of current methods being indirect and based on a large number of assumptions.

The most extensively used method to test homonymous RI in human subjects was developed in the 1970s and consists of evaluation of RI through its modulation of H-reflexes (Pierrot-Deseilligny & Burke, 2005). The design parallels the original experiments in cats in which Renshaw cell activation by antidromic motor axon volleys was shown to modulate motoneuron responses to monosynaptic Ia afferent inputs evoked by stimulating cut dorsal roots (Renshaw, 1946). In humans it is not possible to sever dorsal roots and stimulation is restricted to peripheral nerves. Nerve stimulation results in an orthodromic afferent volley and an antidromic motor axon volley, being the monosynaptic actions of the afferent volley on motoneurons masked by the afterhyperpolarization (AHP) that follows antidromic motoneuron firing, and this cofounds RI actions on Ia synaptic activation of motoneurons. To isolate RI effects, a dual stimulation protocol was developed. In this paradigm, group I afferents (Ia and Ib) are stimulated first (S1) by a low intensity stimulus that does not reach motor axon activation threshold. The afferent volley activates a population of motoneurons and their coupled Renshaw cells inside the spinal cord (causing an H1 conditioning reflex). This is followed by a second supramaximal nerve stimulation (SM) to recruit all of the motor pool (in addition to group I afferents) causing maximal muscle activation. If SM follows S1 by a short interval, the antidromic motor axon volley collides with the H1-reflex and this does not reach the muscle. Importantly, H1-reflex-activated motoneurons are not invaded by SM-generated antidromic action potentials and therefore are released from the spike AHP. In these conditions the effect of RI following H1 can be tested on a second reflex, H′, caused by the SM-evoked afferent volley in the absence of antidromic motoneuron firing. Since the H′ reflex can only activate H1-reflex-activated motoneurons (i.e. lacking antidromic AHPs), H′ should be equal to or smaller than H1. Increasing the intensity of H1 causes first a parallel increase in H′ (because more motoneurons are available to be recruited by the SM afferent volley) until a high enough H1 is evoked, after which H′ diminishes in amplitude relative to H. This decline is interpreted as the effect of RI on motoneuron recruitment when all conditions and assumptions are confirmed. One important piece of evidence demonstrating that H′ modulation is indeed due to RI is its specific potentiation (reductions in H′ without affecting H1) with L-acetylcarnitine, a compound that enhances transmission at cholinergic synapses between motoneurons and Renshaw cells (Mazzocchio & Rossi, 1997).

However, interpretation of results is sometimes disputed based on differences in technique and consideration of underlying assumptions. One problem is that voluntary contractions would cause a descending-driven motor volley that can collide with SM and thus H1 and H′ recruited motoneurons might not be necessarily the same, as is required for correct interpretation of results (Grospretre *et al.* 2016). Another is that when comparing different conditions, the size of H1 should be ideally very similar, assuming AHP and motoneuron excitability are relatively constant, but this is difficult to defend nowadays when so many motoneuron properties are known to vary with functional state and disease. Moreover, H-reflexes can also be diminished in patients by direct effects of the disease on Ia afferents. A further confounding factor is the contribution of Ib inputs. Ib inhibition of homonymous motoneurons is avoided by timing SM 10 ms after S1; however, Ib inputs can also contribute to reduce the size of the SM-evoked H′, and spinal Ib pathways gains are known to be adjusted rapidly in different conditions. Testing RI duration by varying S1-SM intervals is also imperfect because SM needs to be evoked before the H1 reflex passes the location of nerve stimulation, imposing an upper limit of approximately 35 ms (depending on nerve length and conduction velocity) that is shorter than RI duration in experimental animals. The method is also restricted to muscles where robust H-reflexes can be elicited and reportedly only 65% of normal subjects display H′ reflexes (Pierrot-Deseilligny & Burke, 2005). All these problems confound outside readers

 DOI: 10.1113/JP277715

of the human literature (like me) but should by no means be construed as implying that the method is not useful. On the contrary, the paired H-reflex has provided a wealth of data on homonymous RI in normal subjects and in patients (Pierrot-Deseilligny & Burke, 2005), with reports going to great lengths to corroborate assumptions and refine interpretations. Yet it seems beneficial to develop more direct methods that overcome limitations and avoid estimating RI through effects on different circuitries that might or might not be altered in disease.

In this issue of *The Journal of Physiology*, Özyurt and colleagues from Kamal Türker's lab (Özyurt *et al.* 2019) propose a method that, although more complex to implement, offers simpler interpretation of results and relies on fewer assumptions. If further validated for other muscles and conditions the technique could be applied when the paired H-reflex method is not possible, as well as ratifying conclusions using the paired H-reflex. The focus on single motor unit modulation rather than population responses also opens the possibility of gaining knowledge on the distribution of RI throughout the motor pool, particularly applying new technological advances for sampling large populations of motor units (Farina *et al.* 2016). This question has dragged on experimentally but is critical to understanding the effects of RI on motor unit recruitment and how this changes with motor task and disease.

The method is based on testing RI over the voluntarily evoked background discharge of single motor units with stimulations adjusted to elicit antidromic spikes in the faster motor units and RI effects measured on the discharge of slower motor units, thus preventing contamination by antidromic AHPs. Multiple single motor units are isolated and analysed in peristimulus time histograms (PSTHs) and peristimulus firing frequencygrams (PSFs) with the corresponding cumulative sums. Similar approaches have been used before, mainly to study heteronymous RI in conjunction with the paired H-reflex, but were seldom used for homonymous RI. The refinements introduced allowed the authors to demonstrate homonymous RI in soleus motor units and accurately describe its full duration (>50 ms) and how this varies with motor unit firing frequency and motor unit size. The technique is, of course, not without limitations. For one, its design implies that RI can only be tested (as acknowledge by the authors) from the largest to smaller motor units and not vice versa. Moreover, testing was done on tonic soleus activation during sustained plantar flexion. This allowed estimates of statistically significant changes in PSTHs and PSFs compared to steady-state tonic firing before the stimulus. It has yet to be shown whether the technique will be useful during behaviours with continual modulation of motor unit firing. Nevertheless, this technique is a much needed step forward, expanding the range of new methods that will provide further insights into the organization of homonymous RI in human subjects and also help confirm conclusions based on the paired H-reflex technique.

References

Farina D, Negro F, Muceli S & Enoka RM (2016). Principles of motor unit physiology evolve with advances in technology. *Physiology (Bethesda)* **31**, 83–94.

Grospretre S, Duclay J & Martin A (2016). Assessment of homonymous recurrent inhibition during voluntary contraction by conditioning nerve stimulation. *PLoS One* **11**, e0167062.

Mazzocchio R & Rossi A (1997). A method for potentiating Renshaw cell activity in humans.*Brain Res Brain Res Protoc* **2**, 53–58.

Özyurt MG, Piotrkiewicz M, Topkara B, Weisskircher H-W & Türker KS (2019). Motor units as tools to evaluate profile of human Renshaw inhibition. *J Physiol* **597**, 2185–2199.

Pierrot-Deseilligny E & Burke D (2005). *Recurrent Inhibition. The Circuitry of the Human Spinal Cord*, pp. 151–196. Cambridge University Press, New York, USA.

Renshaw B (1946). Central effects of centripetal impulses in axons of spinal ventral roots. *J Neurophysiol* **9**, 191–204.

Additional information

Competing interests

No competing interests declared.

Author contributions

Sole author.

Funding

HHS NIH National Institute of Neurological Disorders and Stroke (NINDS): R01 NS046357 and R21 NS106209.