



Response properties of spinal interneurons in awake, behaving primates

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Abstract

This paper reviews studies on spinal interneurons in awake, behaving monkeys inspired by the work of Prof Patrick D. Wall. Early studies documented the sensory responses of spinal interneurons in unanesthetized monkeys to natural cutaneous and proprioceptive stimulation. More recently, cervical interneurons were documented in monkeys performing an active step-tracking task. During alternating wrist movements, most task-related interneurons showed bidirectional activity, firing during both flexion and extension (in surprising contrast to the unidirectional activity of muscles and corticomotoneuronal cells). Premotor interneurons were identified by post-spike effects in spike-triggered averages of forelimb muscle activity. The cells' post-spike effects were generally congruent with their activity in their preferred direction, although many fired during components of movement when their output effects would seem inappropriate. In an instructed delay period task many interneurons showed preparatory delay period activity, much like cortical neurons. Other studies tested the excitability of corticospinal axons to electrical stimulation and demonstrated both post-spike and task-related modulations in excitability. Together, these studies suggest that many behavioral functions of spinal interneurons remain to be revealed by recording their activity in awake, behaving animals. © 1999 International Association for the Study of Pain. Published by Elsevier Science B.V.

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1. Introduction

A cross-section of the spinal cord reveals a condensed version of the entire nervous system. Sensory input from peripheral receptors arrives via afferent fibers in the dorsal root and dominates the responses of cells in the dorsal horn (Wall, 1967); the motor output is delivered by motoneurons, via the ventral roots, to muscles. Between input and output the intermediate layers of the spinal cord represent association networks that mediate complex and variable sensorimotor connections. Functionally defined areas of the cerebral cortex are similarly specialized for sensory, motor and associational processing. Recording the activities of cortical neurons in behaving monkeys has revealed a rich capacity for behavioral coding; the ever-expanding repertoire of behavioral variables found to be represented in cortical neurons reflects the ingenuity of experimentalists to devise ever more complex tests of higher-order representations. In contrast, spinal cord interneurons have been studied almost entirely in anesthetized, spinal or decerebrate animals, typically in relation to their sensory input and their possible role in mediating motor reflexes. As a result, the cerebral cortex is commonly considered to be the place where higher-order sensorimotor computations and cogni-

tive functions are processed, while the spinal cord deals with 'lower-order' peripheral processing. However, spinal interneurons have extensive reciprocal connections with the cerebral cortex (as well as with other supraspinal centers). The possibility that these interconnections could involve spinal interneurons in behavioral roles that are more sophisticated than simple sensory or motor relays has remained largely unexplored. To test these possibilities, one must examine the properties of spinal interneurons in awake animals under appropriate behavioral conditions. Such behavioral influences on the responses of neurons in the trigeminal nuclei of trained monkeys has already been amply documented in the elegant studies of Dubner and colleagues (reviewed by Dubner and Ren, 1999 this issue).

2. Sensory responses in awake animals

Initial studies of spinal interneurons in intact, awake animals tested their responses to peripheral stimulation. The experimental procedures for acute recording influence the spontaneous and evoked activity of spinal neurons: barbiturate anesthesia depresses spontaneous activity and changes cell responses to natural and electrical stimulation (Wall, 1967; Collins et al., 1995). To circumvent the use of anesthetic agents, decerebrate or spinal preparations are commonly used; however, the response properties of

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many segmental neurons depend on which descending supraspinal systems remain intact. When decerebrate preparations are made spinal, many dorsal horn cells become less responsive to proprioceptive stimuli and are dominated by cutaneous inputs (Wall, 1967). Recording from unanesthetized, intact animals avoids these problems entirely and permits spinal neurons to be studied in a behavioral context.

Early chronic unit studies found that the passive sensory responses of dorsal horn neurons in awake animals generally resembled those seen in acute preparations, with interesting exceptions. In the rat lumbar dorsal horn the responses of single neurons to fixed electrical subcutaneous stimuli were modulated with behavior: the evoked responses decreased during exploratory movements, eating or drinking, and increased when the rat appeared to attend to the region of the stimulus (Wall et al., 1967). In the cat lumbar dorsal horn, cells had conventional cutaneous receptive fields but exhibited no spontaneous activity (Collins, 1987; Sorkin et al., 1988). Many had low tactile thresholds in the awake cat and developed wide dynamic range response profiles (including multimodal responses) under light barbiturate anesthesia (Collins et al., 1990, Collins et al., 1995). In the awake monkey cervical dorsal horn neurons also showed little spontaneous activity when the monkey was quiescent; their receptive fields generally resembled those previously described in the cat (Courtney and Fetz, 1973; Bromberg and Fetz, 1977). Fig. 1 illustrates the responses and tactile receptive fields of cervical interneurons in a passive, awake monkey. Of the 128 adequately tested neurons, one-fifth responded to tactile stimulation, two-fifths were driven by proprioceptive stimulation, and the remaining two-fifths were unresponsive to any stimulation. Few interneurons showed convergence of cutaneous and proprioceptive input. The width of the dynamic range that could be tested in awake monkeys was limited at the upper end by the need to maintain unit isolation and keep the subjects cooperative. Nevertheless, the monkeys easily tolerated repeated electrical stimulation of nerves sufficient to evoke reflex responses and test latencies. When the monkeys performed a step-tracking task that required alternating movements of the wrist, the responses of many interneurons were modulated more intensely during active than passive movements.

3. Movement-related activity of interneurons

The possible motor function of spinal interneurons has been largely inferred from their reflex responses to peripheral and descending input documented in anesthetized animals (reviewed by Baldissera et al., 1981; Jankowska, 1992). Remarkably little is known directly about the role of segmental interneurons in normal movements. Behavioral experiments can elucidate two crucial properties that are essential to understanding the functions of segmental

neurons in movements: their activity patterns during normal voluntary limb movements and their output effects on the agonist muscles. These two properties together provide important information about the contribution of spinal neurons to muscle activity.

To elucidate the role of segmental neurons in voluntary movement, we applied the techniques previously used to investigate supraspinal premotor (PreM) neurons in the motor cortex and red nucleus of nonhuman primates (reviews: Cheney et al., 1988; Fetz et al., 1989). Spike-triggered averages (STAs) of muscle activity during active movement have revealed the post-spike effects of PreM neurons on their target muscles (Fetz and Cheney, 1980; Kasser and Cheney, 1985; Lemon et al., 1986; Mewes and Cheney, 1991). The activities of these PreM cells during the performance of a ramp-and-hold motor task exhibited characteristic discharge patterns that reveal their control of dynamic and static components of force (Cheney and Fetz, 1980; Buys et al., 1986; Cheney et al., 1988; Fetz et al., 1989). We investigated these same properties for dorsal root afferents (Flament et al., 1992) and segmental interneurons (Fetz et al., 1996; Perlmutter et al., 1998; Maier et al., 1998) during a similar step-tracking task. Comparing the discharge patterns and post-spike effects of supraspinal and segmental

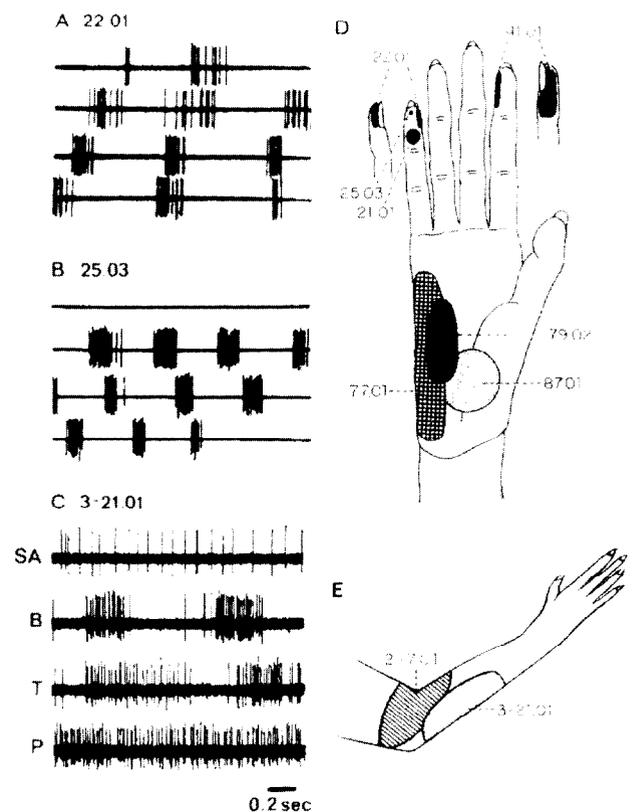


Fig. 1. Responses of cervical interneurons to cutaneous stimulation in awake monkey. A–C: Responses of labeled units to touch over receptive fields illustrated in D–E. Traces in C show spontaneous activity (SA) and responses to brush (B), touch (T) and pinch (P). (From Bromberg and Fetz, 1977.)

PreM neurons elucidates their relative contributions to voluntary muscle activity.

Activity of units in the cervical spinal cord was recorded with moveable tungsten microelectrodes (Perlmutter et al., 1998). Electromyographic (EMG) activity from wrist and digit flexor and extensor muscles was recorded with implanted multi-stranded wires. During recording sessions the monkeys made ramp-and-hold torque responses of the wrist in an isometric step-tracking task. (All our surgical and behavioral training procedures were approved by the Animal Care and Use Committee at the University of Washington. The animals were cared for by the veterinary staff of the Regional Primate Research Center and housed in compliance with NIH standards as stated in the *Guide for the Care and Use of Laboratory Animals* (DHEW Publication NIH85-23, 1985) and with recommendations from the Institute of Laboratory Animal Resources and the American Association for Accreditation of Laboratory Animal Care.)

4. Output effects from spinal interneurons

Interneurons coactivated with muscles during the step-tracking task were tested for correlational linkages with muscles by compiling STAs of full-wave rectified EMG activity. Most of the interneurons ($414/575 = 72\%$) showed no spike-related changes; otherwise, these 'unidentified'

interneurons had response properties resembling those of premotor interneurons (PreM-INs). One hundred interneurons were identified as PreM-INs on the basis of post-spike facilitation or suppression with onset latencies of ≥ 3.5 ms after the trigger spike (derived from the minimal latency of post-stimulus effects evoked by single-pulse microstimuli delivered through the spinal recording electrode). In some cases, changes in the level of averaged EMG activity in the STA started before or near the trigger point. These early changes could be due only to synchronous activity in other PreM units that fired before the triggering neuron. STA features were predominantly facilitatory (85%) and occurred twice as often in flexor as in extensor muscles. Fig. 2 illustrates the STAs and response averages for one of the rare inhibitory PreM-INs. This cell in segment C_8 fired while the monkey generated both flexion and extension torques, with a higher level of activity during the flexion hold than during extension (response average at right). A transient pause accompanied the dynamic transitions between extension and flexion holds. The STAs (left) reveal that this neuron produced post-spike suppression in two of the co-activated flexor muscles.

Two-thirds of the PreM-INs had post-spike effects in only one of the recorded muscles; the remaining third affected multiple target muscles. The spinal PreM-INs had more restricted muscle fields than did corticomotoneuronal and rubromotoneuronal cells. Moreover, PreM-INs rarely

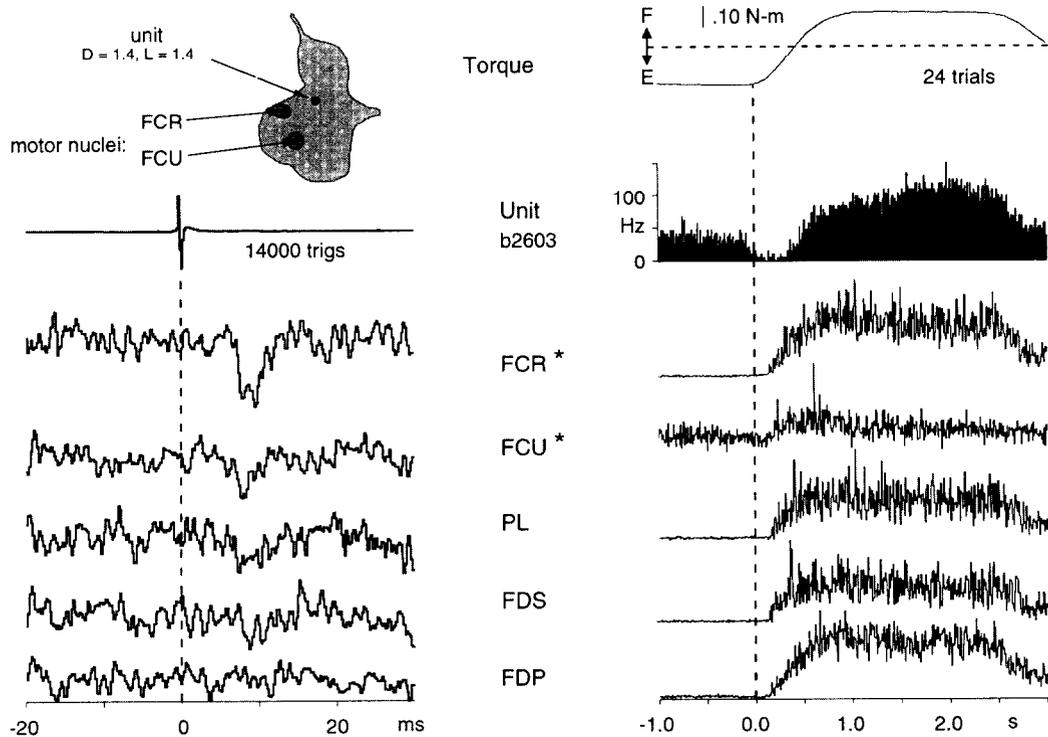


Fig. 2. STAs and response averages of a flexor-related inhibitory PreM-IN. This neuron produced significant postspike suppression in the flexor carpi radialis and ulnaris (FCR and FCU) muscles (*), in the STA of rectified EMG activity (left). The weak suppressions seen in palmaris longus (PL) and flexor digitorum sublimis (FDS) were not statistically significant. The cell's location was estimated on the basis of microelectrode coordinates (inset). The response average (right) shows higher activity during flexion than extension and a pause during dynamic transition in torque direction. (From Fetz et al., 1996.)

exerted reciprocal effects on antagonist muscles. This would indicate that supraspinal PreM cell activity tends to produce a coordinated activation of muscle groups, whereas PreM-INs tend to facilitate or suppress specific muscles.

5. Response patterns of spinal interneurons

Although post-spike effects identify the target muscles affected by a PreM neuron, the magnitude of the cell's effect on target motoneurons during active movement is proportional to its discharge rate. Most of the PreM-INs showed tonic or phasic-tonic activity associated with the ramp-and-hold torque trajectory in their preferred direction. Three-fourths of the interneurons were active for both directions of movement, in contrast to the strictly unidirectional activity of corticomotoneuronal cells, afferent PreM fibers and motor units. Most of the interneurons were activated more during movements in their preferred direction, but also had some activity during movement in the opposite direction. Thus, many interneurons that facilitated primary flexor or extensor muscles continued to fire when their target motor units became silent. A few PreM-INs had a steady discharge rate that was not modulated during alternating flexion and extension torques, similar to the previously reported unmodulated rubromotoneuronal cells (Mewes and Cheney, 1991).

The post-spike effects of most PreM-INs were congruent with their discharge patterns (e.g. they facilitated the muscles active in their preferred direction). However, many interneurons showed activity during phases of movement in which their output effects would be inappropriate. The unidirectional activation of corticomotoneuronal cells suggests that they are activated under relatively particular movement conditions, whereas spinal PreM-INs are recruited more generally, with excitatory and inhibitory interneurons coactivated in an overlapping fashion. Relatively few PreM-INs had incongruous relationships between activation and post-spike effects. For example, the cell in Fig. 2 suppressed the muscles it was most strongly coactivated with (a pattern that might be expected for Renshaw cells).

6. Delay period activity in interneurons

To investigate the possible involvement of spinal interneurons in early stages of movement preparation we recorded their activity in two monkeys while they performed an instructed delay task (Prut et al., 1998; Prut and Fetz, 1999). Trials included a rest period (monkey holding a cursor in a central target zone corresponding to zero torque), an instructed delay period (beginning with a transient cue that indicated the direction of the next movement and ending with a go signal for movement), and an active torque hold period (during which the monkey acquired the remembered target). A third of the spinal interneurons

showed significant delay period modulation (SDM) relative to the precue rest period. There was no detectable EMG activity during the delay. For some interneurons the change in firing rate during the instructed delay was in the same direction as the activity change during the subsequent movement, as would be expected if preparatory activity were simply a subthreshold version of the movement activity. However this was not the case for most interneurons. Many showed changes only during the delay, or only during movement, but not both. Of the interneurons that showed changes in both, firing rates during the delay and torque periods were modulated in the opposite direction (i.e. increase vs. decrease) for 39% of interneurons with SDM. Two-thirds of interneurons with SDM were inhibited during the delay, irrespective of the direction of change of firing rates during movement. These results indicate that spinal circuitry is involved in the earliest stages of movement preparation, in a manner similar to motor and premotor cortical areas. In terms of motor preparation, these delay period activities can be understood as reflecting two overlapping processes: a subthreshold preparation for the required movement, superimposed on a general inhibition of overt expression of this activity until the go signal. These first observations of spinal cord neurons in behaving animals raise the intriguing possibility that other 'higher-order' representations might be found at the spinal level.

7. Excitability changes in corticospinal axons

In addition to documenting responses of neurons during voluntary movement we also investigated whether the conduction of corticospinal activity could be presynaptically modulated by changes in excitability of pyramidal tract axons (Schmied and Fetz, 1987). As is well known, descending pathways control sensory input at the earliest level through presynaptic inhibition of afferent fibers (Wall, 1964, 1967; Fetz, 1968; Baldissera et al., 1981; Jankowska, 1992). A convenient and versatile way to document the depolarization of terminals associated with presynaptic inhibition is to test their excitability through direct electrical stimulation (Wall, 1958). The excitability test can also be used to determine whether descending fibers themselves are depolarized. Early studies showed that axonal excitability of corticofugal neurons was transiently increased by peripheral stimulation in anesthetized animals (Dubner and Sessle, 1971; Mann et al., 1977; Rudomin et al., 1978; Mann and Follett, 1982). This increased excitability was thought to reflect an axonal depolarization. However, the electrical threshold of an axon also changes after conduction of an action potential (Raymond, 1979; Swadlow et al., 1980). Following an immediate refractory period is a period of increased excitability lasting 10–50 ms. Since peripheral stimulation often evokes responses in the tested neurons, it was important to distinguish these two mechanisms.

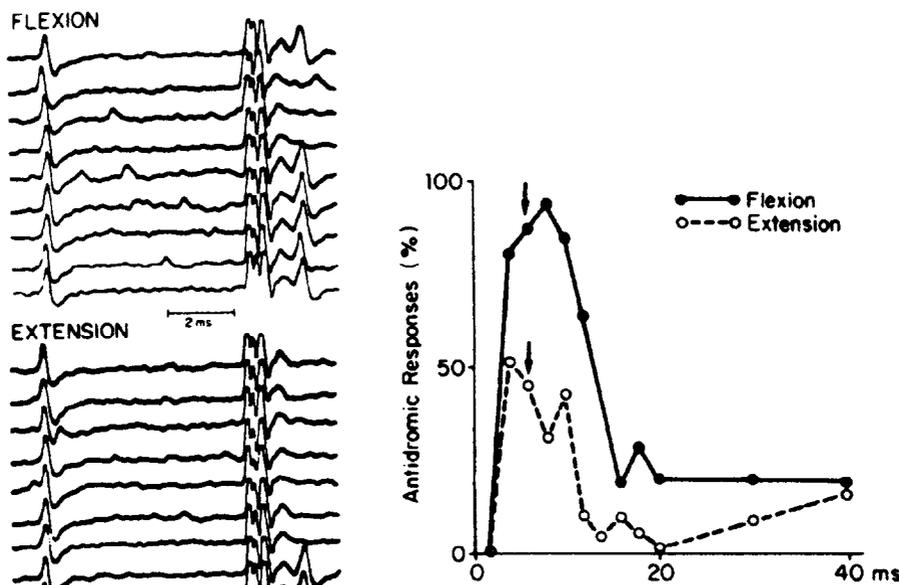


Fig. 3. Changes in axonal excitability of a pyramidal tract neuron. Left: Response rasters show spontaneous spikes followed, after fixed latency, by constant-intensity PT stimuli, some of which evoked antidromic action potentials. The probability of antidromic response was greater during active wrist flexion than extension. Right: Probability of antidromic responses as function of post-spike delay, plotted separately for identical stimuli delivered during flexion (solid curve) and extension (dashed curve). (From Schmied and Fetz, 1987.)

These mechanisms were investigated in awake, behaving primates by documenting the threshold of pyramidal tract fibers to electrical stimulation in the medullary pyramids. Changes in axonal excitability were found to be a function of the delay after an orthodromic spike as well as of motor activity. The antidromic responses of a pyramidal tract neuron (PTN) to constant-intensity peri-threshold stimuli delivered at a fixed delay after an orthodromic spike are shown in Fig. 3 (left). The probability of responding – i.e. axonal excitability – was greater during the flexion hold than during extension hold. The increase in excitability peaked about 10 ms after a spontaneous spike (plot at right). The time course of this post-spike increase was similar for threshold tests during wrist flexion and extension, although the absolute values were consistently greater during flexion. Such task-related modulation was independent of cell activity; for example, this PTN fired at higher rates during extension. A common mechanism that could explain both the post-spike and task-dependent increases in excitability is a change in extracellular ions, notably potassium (Somjen, 1979; Swadlow et al., 1980; Sykova, 1983). As described previously (Schmied and Fetz, 1987), such changes in axonal excitability could modulate the conduction and post-synaptic efficacy of corticospinal impulses.

8. Concluding comments

Clearly, recordings in the spinal cord of behaving animals will provide rich opportunities to investigate the normal response properties and behavioral functions of segmental interneurons. Many of the functional issues investigated at

cortical levels, such as preparatory delay period activity, can also be studied in spinal interneurons. Moreover, many phenomena documented in anesthetized animals, such as reflex responses and axonal thresholds, can be seen to be modulated with behavior. One could anticipate that future experiments will reveal that spinal cord interneurons not only process peripheral sensory and motor information, but can participate in the entire spectrum of behavioral functions.

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