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CHAPTER 28

Primate spinal interneurons: muscle fields and response properties during voluntary movement

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Introduction

Our current view of the functions of spinal interneurons in generating voluntary movements is based largely on inferences from observations in anesthetized, immobilized animals. Previous studies have elucidated the convergent inputs to interneurons from afferent fibers and from descending tracts (for reviews see Baldissera et al., 1981; Jankowska, 1992). Still, little is known about 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 significant information about how the activity of spinal neurons contributes to muscle activity. Similar studies have elucidated the response patterns and the output effects of supraspinal premotor (PreM) neurons in the motor cortex and red nucleus (reviews: Cheney et al., 1988; Fetz et al., 1989) and dorsal root afferent fibers (Flament et al., 1992). This paper summarizes results of comparable experiments with interneurons in the cervical spinal cord.

To elucidate the role of segmental neurons in voluntary movement, we applied the techniques previously used to investigate supraspinal PreM cells. Spike-triggered averages (STAs) of muscle activity during active movement have revealed output connections of neurons in the motor cortex and red nucleus to alpha-motoneurons (Fetz and Cheney, 1980; Lemon et al., 1986; Mewes and Cheney, 1991). The activities of these corticomotoneuronal (CM) and rubromotoneuronal (RM) cells during the performance of a simple ramp-and-hold motor task exhibit characteristic discharge patterns that provide insight into the control of dynamic and static components of force (Cheney and Fetz, 1980; Buys et al., 1986; Cheney et al., 1988; Fetz et al., 1989; Mewes and Cheney, 1994). Here, we describe properties of primate segmental interneurons during a similar step-tracking task. Information on the discharge pattern and post-spike effects of interneurons enables us to contrast the relative contributions of supraspinal and segmental PreM neurons to voluntary muscle activity.

The methods for recording activity of units in the cervical spinal cord with movable tungsten microelectrodes have been described elsewhere (Perlmutter et al., 1998). Electromyographic (EMG) activity from wrist and digit flexor and extensor muscles was recorded with multi-stranded wires implanted trans- or subcutaneously. During recording sessions the monkeys made ramp-and-hold torque responses of the wrist in an isometric step-tracking task.

STAs of full-wave rectified EMG activity (Fetz and Cheney, 1980) were computed to identify

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correlational linkages with muscles. PreM-INs produced a post-spike effect in EMG, defined as a change in the level of the averaged EMG activity beginning at least 3.5 ms after the trigger spike (from the minimal latency of post-stimulus effects evoked by single-pulse microstimuli delivered through the spinal recording electrode). PreM-INs could produce either post-spike facilitation (PSF) or post-spike suppression (PSS) or both. The cell's muscle field was defined as the set of muscles exhibiting post-spike effects (after elimination of potentially redundant recordings via electrical cross-talk). In some cases, changes in the level of averaged EMG activity 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 and were termed synchrony effects.

Response patterns of the units during ramp-and-hold torque trajectories were determined from averages aligned at onset of the torque ramps. These response averages included time histograms of the unit activity, averages of rectified EMG activity, and the isometric torque.

Output effects from spinal interneurons

Activity of 575 neurons was recorded in the C6-T1 spinal segments in three macaques while they performed isometric flexion-extension torques about the wrist. These cells were encountered throughout the gray matter, but most were in the intermediate and ventral laminae. Twenty-nine neurons were identified as motoneurons, on the basis of their low firing rates, non-zero recruitment threshold, and depth from the cord dorsum. STAs of unrectified EMGs from these motoneurons often revealed characteristic motor unit profiles in a single forearm muscle after a few hundred triggers. Their response patterns during this task resembled those of peripherally recorded motor units documented more extensively in a previous study (Palmer and Fetz, 1985).

One hundred interneurons exhibited PSF or PSS in at least one muscle beginning at latencies of $\geqslant 3.5$ ms and were classified as premotor interneurons (PreM-INs). In addition, 32 interneurons exhibited synchrony effects without identifiable post-spike effects in any of the recorded muscles (classified as Sy-INs). (25 of the 100 PreM-INs also showed superimposed synchrony and post-spike effects.) Most of the interneurons (414 of 575) showed no spike-related changes and were called unidentified interneurons (U-INs).

Figure 1 shows the STAs and response averages for an excitatory PreM-IN, estimated to reside in lamina VII of the caudal C_8 segment. This PreM-IN discharged tonically during static torques in both directions, with a higher rate during flexion, as shown by the response average (right). This neuron produced PSF in both the pronator teres (PT) and flexor carpi ulnaris (FCU) muscle (left). Of the 100 PreM-INs, 82% showed PSF of target muscles, either in isolation (as in Fig. 1) or superimposed on synchrony facilitation. The mean onset latency of the PSF was 7.3 ms ($n = 137$). The average mean percent increase of PSF above baseline (after synchrony was discounted) was 4.6%.

An inhibitory PreM-IN in segment C_8 is shown in Fig. 2. The STAs reveal PSS in two of the co-activated flexor muscles, flexor carpi radialis (FCR) and FCU. This cell also fired while the monkey generated both flexion and extension torques, with a higher level of activity during the flexion hold than during extension. A transient pause accompanied the dynamic transitions between extension and flexion holds. Fourteen (14%) of the PreM-INs showed only PSS of target muscles. The mean latency of the PSS was 8.5 ms ($n = 24$) and the average mean percent decrease relative to baseline was 4.2%. An additional 4 PreM-INs showed reciprocal effects: PSF of some muscles and PSS of their antagonists.

Surprisingly, post-spike and synchrony effects were about twice as common in flexor muscles as in extensors: 58% of PreM-INs had post-spike effects in flexor muscles only, while 29% had effects in extensors alone and 11% had effects in both. These proportions were similar for both PSF and PSS.

The number of muscles affected by PreM-INs was relatively restricted. Sixty-four of the 100 PreM-INs had post-spike effects in only one of the recorded muscles; the rest had larger muscle fields. On average, post-spike effects were distributed to 33% of the independent co-active muscles (after eliminating potentially redundant recordings). The PreM-INs with divergent post-spike effects in more than one muscle typically affected either flexor or extensor muscles, but not both (25/36 = 69%).

Response patterns and output effects of spinal interneurons

Most of the PreM-INs showed tonic or phasic-tonic activity associated with the ramp-and-hold torque trajectory in their preferred direction. Figure 3 summarizes the responses of various classes of spinal neurons, and also shows the patterns of other PreM cells in their preferred direction. All populations included phasic, tonic and phasic-tonic cells, in varying proportions. Interestingly, the relative proportions of response patterns were generally similar among the three groups of interneurons, indicating no clear preferential patterns for those with functional linkages to muscles.

In contrast to CM cells, afferent PreM fibers and motor units, only one-fourth of the interneurons were active for one direction of movement only. Most of the interneurons were activated more during movements in their preferred direction, but also had some activity during movement in the opposite direction.

A basic functional question concerns the relationship between the post-spike output effects of a PreM-IN and its response patterns relative to its target muscles. These relations are tabulated in Fig. 4, which shows the distribution of post-spike effects to 'agonist' muscles, activated in the cell's preferred direction, and to the 'antagonist' muscles. For some PreM-INs this relationship was totally 'congruent': the postspike effects and activation patterns were functionally completely consistent with simple reciprocal control of flexion/extension movements. Such was the case for the 24 PreM-INs that facilitated agonist muscles and fired only when these muscles were active (Fig. 4). All the other PreM-INs had more 'complex' relations: they showed some degree of 'inappropriate' activity, in which the postspike effect would seem counterproductive. The circled numbers in Fig. 4 indicate interneurons that had postspike effects that were partially consistent with their activation – for example, those that facilitated agonist muscles but were also active during antagonist muscle activity. The cells denoted by triangles had largely paradoxical relations. For example, the inhibitory IN in Fig. 2 increased its activity more during flexion torques and produced PSS in flexor muscles. A few PreM-INs had a steady discharge rate that was not

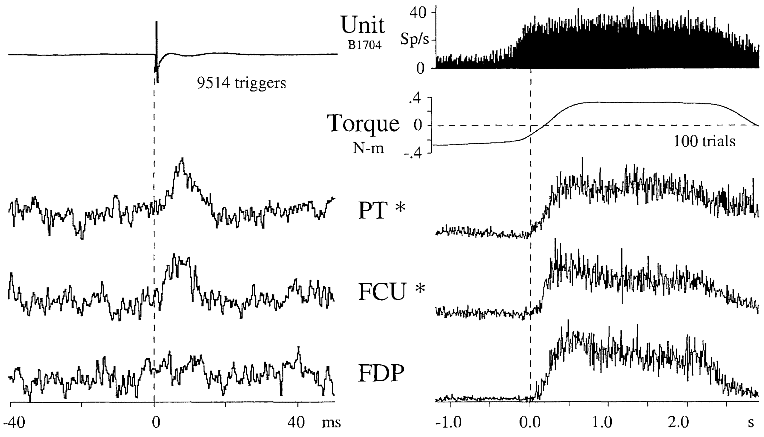


Fig. 1. STAs and response averages of a facilitatory PreM-IN located in lamina VII of caudal C8. The STAs (left) show the triggering action potential (top) and average of rectified EMG in 3 independently recorded co-active muscles. Asterisks indicate muscles with significant post-spike effects. The STAs are plotted with the variances in baseline activity normalized to the STA of the muscle with the largest post-spike effect (PT). The average activity of the neuron during flexion torques is shown in the top right, above the average torque trajectory (positive torque in flexion, negative torque in extension) and average responses of the co-active muscles. This neuron exhibited tonic firing during static torques in both directions, with higher rates for flexion. (From Perlmutter et al., 1998).

modulated during alternating flexion and extension torques, similar to the previously reported unmodulated RM cells (Mewes and Cheney, 1991).

Functional classes of interneurons

These studies have documented the response properties and output effects of segmental interneurons during voluntary motor responses in the primate. Of course, they immediately raise questions about the identity of these interneurons, as conventionally defined by criteria used in acute experiments. Unfortunately, our ability to evoke reflex responses in awake monkeys was limited by our desire to maintain unit isolation and to avoid aversive stimulation. Although our interneurons were not identified in terms of the classical reflex categories, their responses during movement and their output effects on muscles are two important features relevant to understanding spinal control of voluntary movement. Moreover, these two properties were also characterized in other populations of PreM neurons under similar experimental conditions, allowing us to compare the functional properties of spinal and supraspinal PreM populations.

Comparison of post-spike effects of PreM neurons

The post-spike effects of PreM neurons are probably mediated by a monosynaptic linkage to motoneurons (Cope et al., 1987) although polysynaptic relays could also contribute, as previously discussed (Watt et al., 1976; Fetz and Cheney, 1980; Perlmutter et al., 1998). The onset latencies of post-spike effects from different PreM populations are generally consistent with the differences

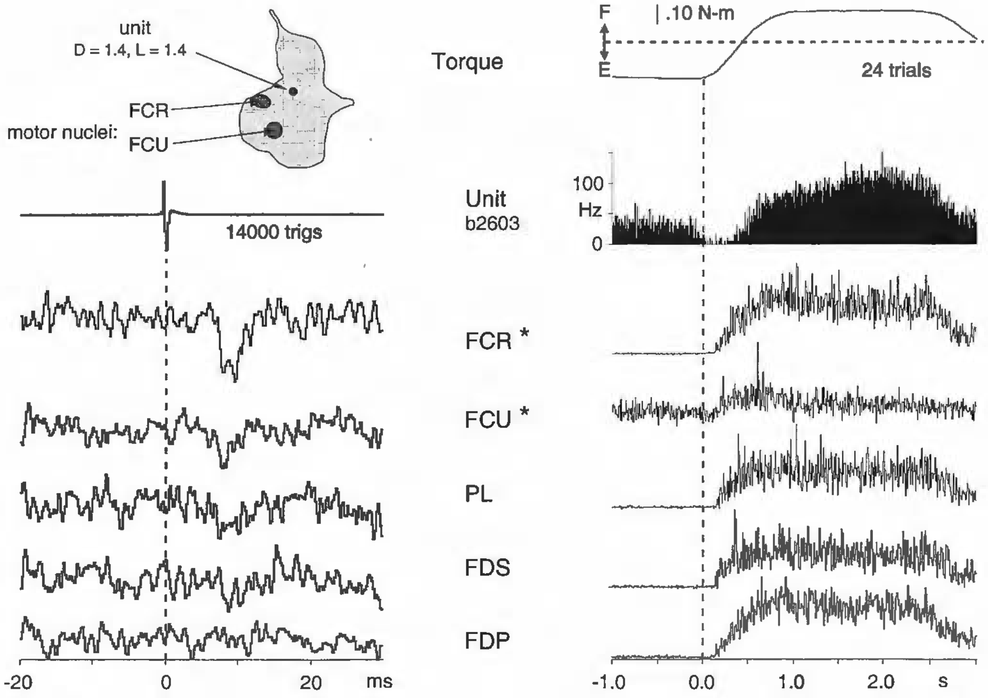
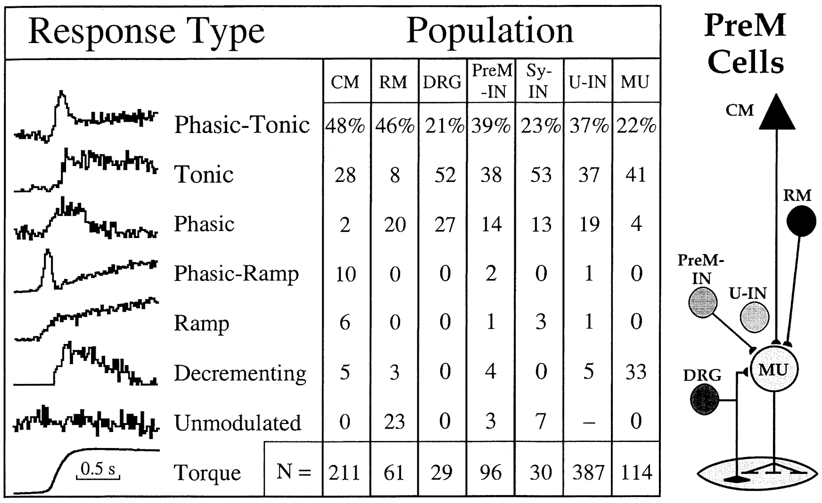
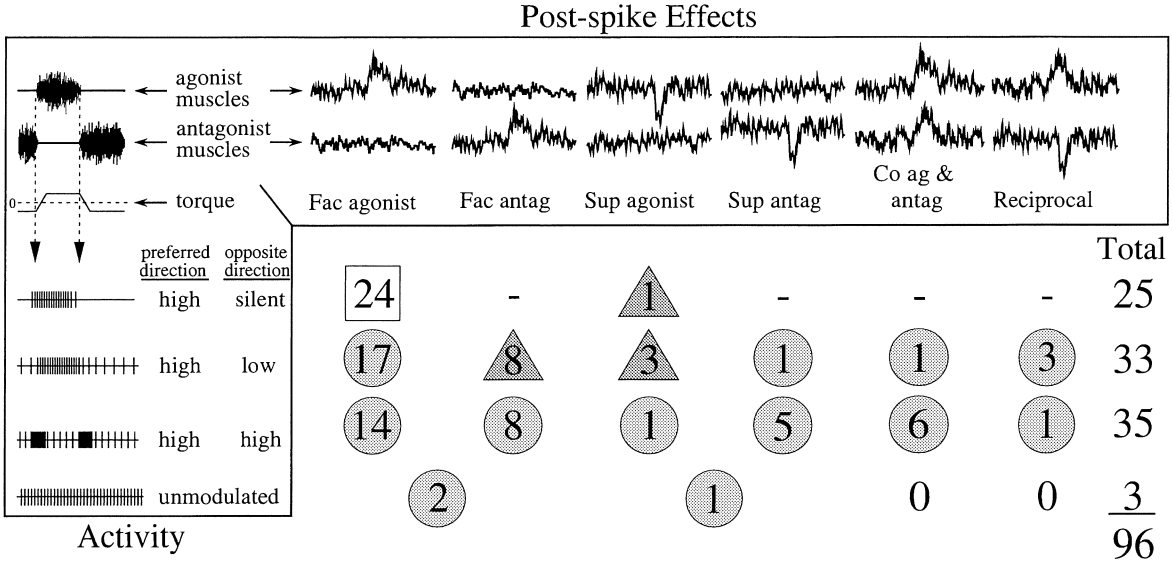


Fig. 2. STAs and response averages of a flexor-related inhibitory PreM-IN. This neuron produced significant postspike suppression in the FCR and FCU muscles (asterisks), in the STA of rectified EMG activity (left). The weak suppressions seen in PL and FDS were not statistically significant. The cell's location was estimated on the basis of microelectrode coordinates (inset). For the locations of cervical motoneuron pools, see Jenny and Inukai (1983). 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).



Response Type	CM	RM	DRG	PreM-IN	Sy-IN	U-IN	MU
Phasic-Tonic	48%	46%	21%	39%	23%	37%	22%
Tonic	28	8	52	38	53	37	41
Phasic	2	20	27	14	13	19	4
Phasic-Ramp	10	0	0	2	0	1	0
Ramp	6	0	0	1	3	1	0
Decrementing	5	3	0	4	0	5	33
Unmodulated	0	23	0	3	7	–	0
Torque N =	211	61	29	96	30	387	114

Fig. 3. Summary of response patterns in the preferred direction for different populations of neurons during generation of flexion and extension torques at the wrist. Examples of each pattern are illustrated on left, and schematic of populations on right. Proportions are given for corticomotoneuronal (CM; Fetz et al., 1989) and rubromotoneuronal cells (RM; Mewes and Cheney, 1991), premotor afferents in dorsal root ganglia (DRG; Flament et al., 1992), spinal premotor interneurons (PreM-IN), spinal unidentified interneurons (U-IN), spinal interneurons with synchrony effects (Sy-IN) and motor neurons (MU) (Maier et al., 1998). The latter combines motor unit data from Palmer and Fetz (1985) with putative motoneurons from present study. Unmodulated U-INs are not included because they were not studied systematically and their proportion could be made arbitrarily large.



preferred direction	opposite direction	Fac agonist	Fac antag	Sup agonist	Sup antag	Co ag & antag	Reciprocal	Total
high	silent	24	-	1	-	-	-	25
high	low	17	8	3	1	1	3	33
high	high	14	8	1	5	6	1	35
unmodulated		2		1		0	0	3
								96

Fig. 4. Relation between response patterns and post-spike effects of PreM-INs. The neuron's 'agonist' muscles are those active in the cell's preferred direction, the direction of torque for which the neuron has the largest increase in activity. Fac, facilitatory effects; Sup, suppressive effects; dashed lines, connections that are not detectable by spike-triggered averaging; agonist and antagonist are not defined for unmodulated neurons. Square, circles and triangles indicate increasing incongruity between response pattern and post-spike effects. (From Perlmutter et al., 1998).

in conduction times, as discussed in more detail elsewhere (Fetz et al., 1989; Perlmutter et al., 1998). The magnitude of the PSF, as quantified by the mean change as a percentage of the pre-trigger baseline EMG activity, was generally similar across populations. Excitatory PreM-INs produced PSFs that were comparable in magnitude to those produced by CM cells (Kasser and Cheney, 1985) and by afferents in the dorsal root ganglion (DRG) (Flament et al., 1992), and slightly larger than those produced by RM cells (Mewes and Cheney, 1991). On the other hand, post-spike suppressions produced by spinal PreM-INs had a shorter mean latency and a larger mean amplitude than those produced by CM (Kasser and Cheney, 1985) and RM cells (Mewes and Cheney, 1991). This suggests that suppressive PreM-INs were probably last-order inhibitory interneurons.

The muscle fields of PreM-INs (expressed as the proportion of independent muscle recordings exhibiting post-spike effects) was slightly smaller than those of CM and RM cells. More than half of the PreM-INs affected only a single target muscle. The fact that PreM-INs facilitated flexor muscles about twice as often as extensor muscles was unique among the PreM populations. Flexors and extensors were facilitated by nearly equal numbers of PreM afferent fibers. RM cells have the opposite distribution pattern, with effects seen more often in extensor muscles than in flexors (Mewes and Cheney, 1991). CM cells facilitated flexor and extensor muscles in similar proportions, although the largest PSFs were in extensor muscles (Fetz and Cheney, 1980). This suggests that certain muscles are preferentially controlled from supraspinal inputs and others from segmental inputs, a result consistent with the intracellular data of Clough et al. (1968).

Discharge characteristics of premotor neurons

While PSF identifies the target muscles affected by the PreM neurons, the neuron's effect on target motoneurons during active movement is proportional to its discharge rate. Comparing the discharge patterns of populations of PreM neurons and motor units in their preferred direction (Fig. 3) shows that all include phasic, tonic and phasic-tonic neurons. The other response patterns appeared in specific subsets of the PreM groups. A remarkable difference is the degree to which the neurons fired in the non-preferred direction. All motor units and CM cells and most PreM DRG afferents were strictly unidirectional. In contrast, all RM cells and most spinal interneurons showed bidirectional activity. Thus, many interneurons that produced PSFs in primary flexor or extensor muscles continued to fire when their target motor units became silent.

The combined inputs from all PreM populations to forearm muscle motoneurons during the wrist task were dominated by tonic and phasic response components. The tonic discharge pattern most accurately reflects the torque trajectory itself and the level of tonic discharge has been proportional to torque for the CM, RM and PreM-IN populations (Cheney and Fetz et al., 1980; Mewes and Cheney, 1994; Maier et al., 1998). Phasic discharges prior to the onset of movement in PreM cells could contribute to rapid activation of motoneurons. Other patterns of activity also were observed during step-tracking, which could provide additional types of control over the motoneurons. Rubral and spinal PreM neurons with steady, unmodulated bidirectional discharge apparently provide an excitatory bias during both flexion and extension phases of the task.

Contrasting functions of cortical and spinal PreM neurons in movement

The differences in the response properties and projection patterns of CM cells and PreM-INs suggest corresponding differences in their contributions to reciprocal wrist movements. The muscle fields of CM cells were larger, and more often involved reciprocal inhibition of antagonists of the facilitated target muscles, indicating that CM cell activity tends to produce a coordinated activation of muscle groups. In contrast, PreM-INs tend to facilitate or suppress specific muscles. On the other hand, the activation of CM cells was more specific: CM cells fired with flexion or extension, but not both, whereas most spinal interneurons were bidirectionally active. This suggests that CM cells are activated under relatively particular movement conditions, while spinal PreM-INs are recruited more generally, with excitatory and inhibitory interneurons coactivated in an overlapping fashion. Thus CM cells represent more explicitly a pattern of coordinated muscle activity, while spinal interneurons tend to affect particular muscles.

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 performing an instructed delay task (Prut et al., 1998, 1999). Again, the monkeys controlled a cursor position by isometric flexion/extension torques about the wrist. Trials included a rest period (cursor held in a central target zone corresponding to zero torque), an instructed delay period (initiated by a transient cue that indicated the direction of the next movement and terminated by a go signal that indicated the time to begin the movement), and an active torque hold period (when the monkey acquired the remembered target). Many spinal interneurons (123/394 = 31%) showed significant delay period modulation (SDM) relative to the precue rest period – in the absence of any sensory input or motor output. There was no detectable low-level 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 a cell's preparatory activity were simply a subthreshold version of its movement-related activity. However for most interneurons this was not the case. Some showed changes specifically in the delay, but not during movement, and many were modulated during movement without SDM. In addition, for 39% 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). 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 preparation for movement involves extensive modulation of spinal interneurons that cannot be predicted from their responses during active movement. Thus, spinal circuitry is involved in the earliest stages of movement preparation, in a manner similar to motor and premotor cortical areas. Indeed, this set-related activity appears to involve widely distributed neural mechanisms.

The activity of spinal interneurons during an instructed delay period suggests that spinal interneurons can be involved in additional functions beyond simple peripheral sensory or motor events. During the delay interval, after the instructional cue and before the go signal, the changes in IN activity reflect more subtle computations than simple subthreshold changes in the direction required for the upcoming movement. In terms of motor preparation, these changes 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 animal raise the intriguing possibility that other 'higher-order' representations might be found at the spinal level, indicating that cognitive processes could be widely distributed.

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List of abbreviations

IN	interneuron
CM	corticomotoneuronal
RM	rubromotoneuronal
STA	Spike-triggered average
PreM	premotor
EMG	electromyographic
PreM-IN	premotor interneuron
PSF	post-spike facilitation
PSS	post-spike suppression
Sy-IN	synchrony interneuron
U-IN	unidentified interneuron
PT	pronator teres

FCU	flexor carpi ulnaris
FCR	flexor carpi radialis
DRG	dorsal root ganglion
SDM	significant delay period modulation

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