Sensory input to primate spinal cord is presynaptically inhibited during voluntary movement

Kazuhiro Seki1,2, Steve I Perlmutter1 & Eberhard E Fetz1

During normal voluntary movements, re-afferent sensory input continuously converges on the spinal circuits that are activated by descending motor commands. This time-varying input must either be synergistically combined with the motor commands or be appropriately suppressed to minimize interference. The earliest suppression could be produced by presynaptic inhibition, which effectively reduces synaptic transmission at the initial synapse. Here we report evidence from awake, behaving monkeys that presynaptic inhibition decreases the ability of afferent impulses to affect postsynaptic neurons in a behaviorally dependent manner. Evidence indicates that cutaneous afferent input to spinal cord interneurons is inhibited presynaptically during active wrist movement, and this inhibition is effectively produced by descending commands. Our results further suggest that this presynaptic inhibition has appropriate functional consequences for movement generation and may underlie increases in perceptual thresholds during active movement.

Normal motor behavior stimulates peripheral receptors, generating self-induced recurrent activity. For example, moving our limbs produces time-varying afferent input from cutaneous and proprioceptive receptors that is transmitted to the central nervous system (CNS), where it potentially interacts with motor commands and cognitive processes. The extent to which this re-afferent input is incorporated into ongoing motor and sensory processing remains a key issue in understanding mechanisms of voluntary movement and perception.

Movement-induced feedback arrives via afferent fibers that make synaptic contact with so-called first-order ‘relay’ neurons in spinal cord that transmit activity to local neural circuits and to higher centers via ascending pathways. These relay neurons represent one of the first stages at which peripheral input could be modulated, so any task-dependent changes in their responsiveness during normal behavior would have significant consequences. To date, the evidence for such changes is largely indirect. For example, ample evidence indicates that muscular and cortical responses evoked by stimulation of peripheral afferents are modulated during voluntary movement. In humans, cortical potentials evoked by stimulation of skin or peripheral afferents are reduced before and during finger movement, and psychophysical thresholds for detecting tactile stimuli are concomitantly increased. During human locomotion, reflex muscle responses evoked from cutaneous and muscle afferents are strongly modulated in a phase-dependent manner. Because these studies examined overall input-output relations, the site and mechanisms that modulate peripherally evoked sensory and motor responses remain unresolved.

Responses of the relay neurons may be modulated by either presynaptic or postsynaptic mechanisms. Postsynaptic modulation via synaptic inputs would affect the neurons’ responses to many inputs, peripheral and descending, whereas presynaptic inhibition could reduce sensory inputs more selectively because it can modify the efficacy of transmitter release from specific afferents. Presynaptic inhibition operates in various relays of the visual, olfactory and somatosensory systems. It is mainly mediated by axo-axonic GABergic synapses that produce ‘primary afferent depolarization’ (PAD) of the afferent fibers. PAD reduces the amount of transmitter released by action potentials invading the presynaptic terminals, thus reducing the size of responses evoked in first-order and subsequent relay neurons. In the spinal cord, PAD in peripheral afferent fibers is typically evoked experimentally by a synchronous volley in other afferents or in descending pathways.

To date, the degree to which PAD occurs during normal behavior could only be inferred from indirect evidence. Fictive locomotion in immobilized, decerebrate cats is accompanied by phase-dependent modulation of PAD of cutaneous and muscle afferents. During active sleep, PAD in muscle afferents and trigeminal primary afferents is enhanced. These studies suggest that PAD could be dynamically modulated, but its operation has not been studied in awake, behaving animals. Sophisticated reflex testing in humans indicates that a decrement of the monosynaptic reflex at the onset of active sleep is mediated by presynaptic mechanisms, but this evidence is indirect and restricted to muscle afferents. Consequently, the occurrence and role of presynaptic inhibition in voluntary behavior remains to be tested directly in intact, behaving animals.

Using new techniques to record the activity of spinal interneurons in awake behaving primates in combination with nerve cuff electrodes to stimulate and record from a peripheral nerve, we found the most direct evidence to date that presynaptic inhibition operates in a behaviorally relevant manner during voluntary movement.

1Department of Physiology and Biophysics, and Washington National Primate Research Center, University of Washington, Seattle, Washington 98195-7290, USA. 2Department of Integrative Physiology, National Institute for Physiological Sciences, 38 Nishigounaka, Myodaiji, Okazaki, Aichi 444-8585, Japan. Correspondence should be addressed to K.S. (kazuseki@nips.ac.jp).

Published online 16 November 2003; doi:10.1038/nn1154
Presynaptic inhibition reduces afferent input to the primate spinal cord during active voluntary movement, with potential effects on movement control and sensory perception. Moreover, the data suggests that this mechanism is evoked more effectively by motor commands than by peripheral input.

RESULTS
To investigate directly the modulation of sensory input during preparation and execution of normal voluntary movements, we recorded the activity of interneurons in the cervical spinal cord of monkeys performing a wrist flexion–extension task with an instructed delay period (Fig. 1). Monkeys produced torque against an elastic load that returned the hand to a rest position in the absence of active muscle contraction. Throughout this behavior, interneuron responses were evoked by electrically stimulating the superficial radial nerve (SR), which contains only cutaneous afferents.

Modulation of SR-evoked responses
We report results from 46 first-order interneurons (Monkey K, 38; Monkey M, 8), that responded at monosynaptic segmental latencies (<1.5 ms) after arrival of the cord dorsum volley. Mean segmental latency was 0.97 ± 0.08 ms (mean ± s.e.m.), and the mean activation threshold was 1.79 ± 0.11 times the current needed to evoke a threshold afferent volley. The representative cell in Figure 2 showed increased activity during active hand movement in both flexion and extension directions (Fig. 2a,c). In first-order interneurons, much of this movement-related activity could reflect input from peripheral receptors activated during the movement27,28. The short-latency responses to SR stimuli are summarized by the post-stimulus histogram peaks compiled for different phases of the task (Fig. 2b). These responses were reliably evoked during the pre-trial rest period but virtually disappeared when the monkey actively generated dynamic torque (active movement) and decreased slightly relative to rest during production of static torque (active hold). However, the evoked responses did not decrease significantly during return to the rest position (passive movement). The average firing rates for different phases of the task (Fig. 2c) show that interneuron discharge increased during active and passive movements; interneuron activity was larger with the extension movements, which stimulated the receptive fields of SR afferents (the radial side of the dorsum of the hand and distal forearm). In contrast, the responses evoked by SR stimulation decreased significantly only during active movements (Fig. 2c). Mean electromyographic (EMG) activity of agonist muscles was highest for the active movement, and muscle activity was absent during the passive movement (Fig. 2c).

The averaged results for all 38 first-order interneurons in this monkey (Fig. 3) were similar to those in Figure 2. Relative to rest, mean firing rates increased most during active movement in both directions, and they also increased to a similar level during passive movement in flexion trials. In contrast, the magnitude of the SR-evoked response decreased drastically during active movements (both flexion and extension) but did not change significantly during passive movements. It should be noted that 34% of the interneurons included in this comprehensive average did not show significant reductions in responsiveness. We found no significant difference in mean firing rate, onset latency or activation threshold between neurons that exhibited the suppression and those that did not. Data from the second monkey (monkey M; n = 8) confirmed these findings.

Given that SR-evoked responses in these interneurons were depressed during the period when their firing rates were highest, it is conceivable that the interneurons' background activity could reduce evoked responses through increased refractoriness. To test this possibility, we compared the SR-evoked responses that were preceded by background spikes with those that were not. The responsiveness of
To determine whether the reduction of SR-evoked responses was dependent on feedback from movement, we analyzed the time course of the reduction around the onset of EMG activity. A typical example of a first-order interneuron whose response probability (peak area in peristimulus time histogram, PSTH) dropped 400 ms before EMG activity started (P < 0.05) is shown in Figure 4. Of the 25 first-order interneurons that showed a suppression of SR-evoked responses during active movement in monkey K, 14 (56%) showed reduction before EMG response onset. On average, the reduction started 400 ms before EMG onset in this population of interneurons (P < 0.05) or a bimodal test (middle, P < 10^{-2}; bottom, P < 10^{-12}).

Two mechanisms could potentially account for the reduction of the monosynaptic responses to SR stimulation. The evoked responses could conceivably have been reduced by postsynaptic inhibition, but this would be inconsistent with the fact that the interneurons were more active during movements than during rest, reflecting an increase in postsynaptic excitatory drive that would have increased, not decreased, interneuron responsiveness. (It remains conceivable that postsynaptic responses could be reduced at distal dendrites, independently of increased drive at the soma, through ‘remote inhibition’—a possibility that has been proposed but not yet proven in spinal interneurons.) A second explanation for the decrease in evoked responses at a time when the cells were more excitable is a reduction of the efficacy of the afferent volleys by presynaptic inhibition.

Primary afferent depolarization during active movement

To further test whether the afferent fibers underwent presynaptic inhibition, we applied Wall’s excitability test for PAD. PAD is associated with reduced transmitter release from presynaptic terminals and also reduces the threshold of afferent terminals to direct electrical stimulation. This increased excitability of the terminals would produce an increase in the antidromic SR nerve volley evoked by
Together, our observations provide direct evidence that presynaptic inhibition suppresses some cutaneous input to the spinal cord in a behaviorally dependent manner during normal voluntary movements. The monosynaptic responses in active first-order interneurons are reduced simultaneously with increased excitability of the relevant afferent terminals, a combination that implicates presynaptic inhibition. It should be noted that presynaptic inhibition can also occur without PAD (e.g., inhibition mediated by G-protein coupled GABAB receptor16, and shunting produced by increased chloride conductance during the activation of GABAergic synapses9). These

**DISCUSSION**

Together, our observations provide direct evidence that presynaptic inhibition suppresses some cutaneous input to the spinal cord in a behaviorally dependent manner during normal voluntary movements. The monosynaptic responses in active first-order interneurons are reduced simultaneously with increased excitability of the relevant afferent terminals, a combination that implicates presynaptic inhibition. It should be noted that presynaptic inhibition can also occur without PAD (e.g., inhibition mediated by G-protein coupled GABAB receptor16, and shunting produced by increased chloride conductance during the activation of GABAergic synapses9). These
additional presynaptic mechanisms could further account for the reduced monosynaptic responses.

The major sources of presynaptic inhibition are peripheral inputs from afferent fibers and central commands in descending pathways. Peripherally evoked presynaptic inhibition in cutaneous afferents arises primarily from other cutaneous afferents, and partly from secondary muscle spindle and tendon organ afferents, which are activated during both active and passive movements. Presynaptic inhibition of afferents can also be evoked from cerebral cortex and brainstem. Descending commands generating ramp-and-hold wrist movement are typically phasic-tonic, beginning before EMG activity onset, and are relatively less active during passive movement. Consistent with the absence of EMG activity during passive movement, our observations that a partial suppression of evoked responses occurs preferentially during active movements and precedes EMG onset point to a dominant role for descending motor commands in generating the presynaptic inhibition of cutaneous afferents, as compared to peripheral feedback that results from movement. PAD could also be generated by accumulated potassium in the extracellular space, but a recent study suggests that this mechanism has only a minor role, at least for cutaneous afferents.

The fact that the afferent input was inhibited presynaptically indicates that the CNS reduces self-induced input at the earliest...
particular afferents. It may also suggest that descending systems can contribute both to spinal circuitry generating movement and to postsynaptic ascending pathways. During active movements, the perceived intensities of cutaneous reflexes on muscle activity are decreased and perceptual thresholds are reduced during movement. We also observed that the amplitudes of field potentials evoked in primary somatosensory cortex by SR stimulation are modulated in parallel with the responses of the first-order interneurons (data not shown). Of course, these cortical effects could also be mediated by modulation of the direct dorsal column pathway. Primary afferents ending in the cuneate nucleus also undergo presynaptic inhibition evoked from cortex and periphery, which may turn out to be similarly modulated with behavior. Further modulation may occur at other relay sites and within cortical circuits. In any case, the present results indicate that

The sensory consequences of the observed inhibition would arise from contributions of these interneurons to ascending pathways, such as post synaptic axons in the dorsal columns and spinothalamic pathways. During active movements, the perceived intensities of cutaneous stimuli are decreased and perceptual thresholds increased. Moreover, the cortical potentials evoked by cutaneous stimulation are reduced during and before movement. We also observed that the amplitudes of field potentials evoked in primary somatosensory cortex by SR stimulation are modulated in parallel with the responses of the first-order interneurons (data not shown). Of course, these cortical effects could also be mediated by modulation of the direct dorsal column pathway. Primary afferents ending in the cuneate nucleus also undergo presynaptic inhibition evoked from cortex and periphery, which may turn out to be similarly modulated with behavior. Further modulation may occur at other relay sites and within cortical circuits. In any case, the present results indicate that

The segmental motor consequences can be inferred from the muscle responses evoked by electrical microstimulation within the spinal cord. Intraspinal stimulation at sites of first-order interneurons typically produced strong inhibition of task-related EMG in flexor and extensor muscles, as seen in Figure 7a. Thus, output from these sites, which probably reflects the effects of the recorded first-order interneurons and afferent fibers, would reduce ongoing muscle activity. This result is consistent with the known inhibitory effects of cutaneous reflexes on muscle activity. In fact, for 11/38 interneurons, spike-triggered averages of EMG confirmed directly that these interneurons produced post-spike suppression (Fig. 7b). The suppression of these interneuron responses would therefore aid movement generation, as sensory-evoked activation of these interneurons during movement would produce unpredictable inhibition of agonist motor neurons and impede activation of agonist muscles. The dorsum of the monkey’s hand was always in contact with the hand-holder, so presynaptic inhibition would help suppress the inappropriate reflex inhibition of agonists by attenuating input from cutaneous afferents. Thus, presynaptic inhibition of peripheral input during active movement makes functional sense. Moreover, the lack of presynaptic inhibition during the passive movement is also functionally appropriate, as muscle activity is absent during this phase.

Previous studies found that the stimulation of cutaneous pathways and dorsal horn interneurons can have both excitatory and inhibitory actions on cat hindlimb motor neurons. Cutaneous afferents had predominantly inhibitory effects in slow-twitch motor units and excitatory effects in fast-twitch motor units in anesthetized cat and awake human subjects. In our behavioral task, the low levels of wrist torque were probably generated largely by slow-twitch motor units. This could explain why our stimulus- and spike-triggered averages revealed predominantly inhibitory effects (compare to ref. 41).

The sensory consequences of the observed inhibition would arise from contributions of these interneurons to ascending pathways, such as postsynaptic axons in the dorsal columns and spinothalamic pathways. During active movements, the perceived intensities of cutaneous stimuli are decreased and perceptual thresholds increased. Moreover, the cortical potentials evoked by cutaneous stimulation are reduced during and before movement. We also observed that the amplitudes of field potentials evoked in primary somatosensory cortex by SR stimulation are modulated in parallel with the responses of the first-order interneurons (data not shown). Of course, these cortical effects could also be mediated by modulation of the direct dorsal column pathway. Primary afferents ending in the cuneate nucleus also undergo presynaptic inhibition evoked from cortex and periphery, which may turn out to be similarly modulated with behavior. Further modulation may occur at other relay sites and within cortical circuits. In any case, the present results indicate that

The sensory consequences of the observed inhibition would arise from contributions of these interneurons to ascending pathways, such as postsynaptic axons in the dorsal columns and spinothalamic pathways. During active movements, the perceived intensities of cutaneous stimuli are decreased and perceptual thresholds increased. Moreover, the cortical potentials evoked by cutaneous stimulation are reduced during and before movement. We also observed that the amplitudes of field potentials evoked in primary somatosensory cortex by SR stimulation are modulated in parallel with the responses of the first-order interneurons (data not shown). Of course, these cortical effects could also be mediated by modulation of the direct dorsal column pathway. Primary afferents ending in the cuneate nucleus also undergo presynaptic inhibition evoked from cortex and periphery, which may turn out to be similarly modulated with behavior. Further modulation may occur at other relay sites and within cortical circuits. In any case, the present results indicate that

The sensory consequences of the observed inhibition would arise from contributions of these interneurons to ascending pathways, such as postsynaptic axons in the dorsal columns and spinothalamic pathways. During active movements, the perceived intensities of cutaneous stimuli are decreased and perceptual thresholds increased. Moreover, the cortical potentials evoked by cutaneous stimulation are reduced during and before movement. We also observed that the amplitudes of field potentials evoked in primary somatosensory cortex by SR stimulation are modulated in parallel with the responses of the first-order interneurons (data not shown). Of course, these cortical effects could also be mediated by modulation of the direct dorsal column pathway. Primary afferents ending in the cuneate nucleus also undergo presynaptic inhibition evoked from cortex and periphery, which may turn out to be similarly modulated with behavior. Further modulation may occur at other relay sites and within cortical circuits. In any case, the present results indicate that

The sensory consequences of the observed inhibition would arise from contributions of these interneurons to ascending pathways, such as postsynaptic axons in the dorsal columns and spinothalamic pathways. During active movements, the perceived intensities of cutaneous stimuli are decreased and perceptual thresholds increased. Moreover, the cortical potentials evoked by cutaneous stimulation are reduced during and before movement. We also observed that the amplitudes of field potentials evoked in primary somatosensory cortex by SR stimulation are modulated in parallel with the responses of the first-order interneurons (data not shown). Of course, these cortical effects could also be mediated by modulation of the direct dorsal column pathway. Primary afferents ending in the cuneate nucleus also undergo presynaptic inhibition evoked from cortex and periphery, which may turn out to be similarly modulated with behavior. Further modulation may occur at other relay sites and within cortical circuits. In any case, the present results indicate that

The sensory consequences of the observed inhibition would arise from contributions of these interneurons to ascending pathways, such as postsynaptic axons in the dorsal columns and spinothalamic pathways. During active movements, the perceived intensities of cutaneous stimuli are decreased and perceptual thresholds increased. Moreover, the cortical potentials evoked by cutaneous stimulation are reduced during and before movement. We also observed that the amplitudes of field potentials evoked in primary somatosensory cortex by SR stimulation are modulated in parallel with the responses of the first-order interneurons (data not shown). Of course, these cortical effects could also be mediated by modulation of the direct dorsal column pathway. Primary afferents ending in the cuneate nucleus also undergo presynaptic inhibition evoked from cortex and periphery, which may turn out to be similarly modulated with behavior. Further modulation may occur at other relay sites and within cortical circuits. In any case, the present results indicate that
presynaptic inhibition could have an important role in these psychophysical and physiological phenomena.

In summary, we report direct evidence that presynaptic inhibition modulates cutaneous input to the primate spinal cord preferentially during normal voluntary movements. Thus, the central commands initiating movement are accompanied by a reduction of self-induced inputs that could counteract the movement and potentially interfere with accurate control. This presynaptic inhibition also contributes to the increased psychophysical thresholds observed during movement. Similar mechanisms may operate at relays of other sensory modalities during normal behaviors.

METHODS

Subjects. We obtained data from two male *Macaca nemestrina* monkeys (K and M). Experiments were approved by the Institutional Animal Care and Use Committee at the University of Washington. During training and recording sessions, the monkeys sat upright in a primate chair with the right arm restrained and elbow bent at 90°. The hand was held in a cast with the fingers extended and the wrist in the mid-supination/pronation position. The left arm was restrained loosely.

Surgical implant. After training, surgeries were performed aseptically with the animals under 1–1.5% isoflurane anesthesia. Head stabilization lugs were cemented to the skull with dental acrylic and anchored to the bone via screws. A stainless steel recording chamber was implanted over a hemilaminectomy in the lower cervical vertebrae. Bipolar electromagnetic electrodes were implanted subcutaneously in 10–12 forearm muscles. Two cuff electrodes were implanted on the SR nerve: a distal bipolar cuff for stimulation (midway between elbow and wrist) and a tripolar cuff for recording volleys (4–5 cm proximal to the bipolar cuff). The threshold current to evoke an afferent volley (115 ± 14 µA) and the volleys evoked by each intensity were stable throughout the behavioral epochs.

Recording procedure. During recording sessions, the head and vertebral implants were secured to the primate chair and a microdrive was attached to the chamber via an X–Y positioning stage. Activity of neurons in the C6-T1 segments was recorded extracellularly with tungsten microelectrodes while the monkey performed wrist flexion and extension movements in an instructed delay task. The SR nerve was stimulated at 3 Hz during recording sessions, and units with short-latency evoked responses were studied selectively. For testing the modulation of interneuron responses, stimulus current was adjusted so that the probability of evoking a response during task performance was approximately 50%. The segmental response latency was calculated relative to the incoming volley recorded in the cord dorsum potential (CDP) in some recording tracks. We adopted a central latency of less than 1.5 ms as a criterion for monosynaptic linkage, consistent with previous evidence. Immediately after recording SR-evoked responses for some interneurons, we carried out excitability testing without moving the electrode. Intraspinal microstimuli (0.1-ms bipolar pulses, 3–10 Hz, 3–30 µA) were delivered through the microelectrode during task performance, and antidromic compound action potentials were recorded by the proximal SR cuff electrode and then averaged (Fig. 6).

Measurements of antidromic volleys. The sizes of the antidromic volleys in the SR nerve evoked by intraspinal microstimulation were evaluated in terms of their peak-to-peak amplitudes and areas. The bins with maximal (peak) and minimal (trough) amplitudes were first identified from a comprehensive average of the antidromic volleys compiled for all stimuli and all behavioral epochs. This averaged volley was also used to identify the onset and offset bin of the volley, and an inflection bin between its peak and trough, for measurement of the area. The inflection bin was defined by the time that the average waveform crossed the baseline mean, as determined by the average value from 10 to 5 ms before the intraspinal stimulus. These bins were then used to measure the amplitudes and areas of the individual volleys evoked by each stimulus. Peak-to-peak amplitude was measured as the difference between the maximal value in three adjacent bins around the peak bin and the minimal value of three adjacent bins around the trough bin. This measure was used to deal with sampling jitter in individual records. Area was measured by summing the values of each bin from onset to inflection and from inflection to offset, and subtracting the latter from the former. For statistical comparison, amplitude and area were also measured for two baseline intervals before and after the average volley (Fig. 6b) using procedures and bins with relative spacing identical to those used for the volleys. For each behavioral epoch, the amplitudes and areas of antidromic volleys and baselines were statistically compared using an unpaired t-test.

ACKNOWLEDGMENTS

We thank J. Garlid, S. Gilbert, L. Shupe and S. Votaw for technical assistance. This work was supported by National Institutes of Health grants NS 12542, NS 67811 and RR 00106, and Human Frontiers Science Program grant LT0070/1999-B.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

Received 15 September; accepted 15 October 2003

Published online at www.nature.com/natureneuroscience/


