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Evidence for Excitatory Input to Ventral Spinocerebellar Tract Neurons Mediated by Motoneuron Collaterals

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=ABSTRACT=

This study evaluated the hypothesis that motoneuron collaterals modulate the excitability of ventral spinocerebellar tract neurons. In acute cats, 128 ventral cerebellar tract cells were studied extracellularly to determine the effects of ventral root stimuli. The majority of the cells responded to ventral root stimulation with either short or long latency increases in spike discharge. In many cells with sufficient spontaneous activity ventral root stimulation also evoked a long lasting reduction in activity. In preparations with the dorsal root ganglion removed VSCT neurons had similar response properties. In some cells contralateral ventral root stimulation also evoked excitatory responses. These findings indicate the VSCT can provide the cerebellum with information regarding activity in the final output neurons of the motor system, the alpha motoneurons.

Key Words: Ventral spinocerebellar tract, Motoneuron collaterals, Spinal cord, Cerebellar afferents, Spinal pathways

INTRODUCTION

The ventral spinocerebellar tract (VSCT) arising contralaterally from lower thoracic, lumbar, and more caudal segments passes via the ventral funiculus and ascends in the ventrolateral fasciculus (Xu & Grant, 1994). The main portion of the VSCT enters the cerebellum via the superior cerebellar peduncle. A minor portion originating from the sacrococcygeal region enters via the restiform body (Xu & Grant, 1994). Both dorsal spinocerebellar tract (DSCT) and VSCT can transmit exteroceptive information but respond selectively to different features of physiologic stimuli (Kim et al, 1986). The VSCT plays the crucial role in modulating the cerebellar Purkinje cells (Arshavsky et al, 1984b). A hypothesis was proposed that the main content of signals conveyed by the VSCT and spino-reticulo-cerebllar pathway to the cerebellum is the information regarding activity of the generator of rhythmic oscillations that is located in the L3-L5 spinal segments (Arshavsky et al, 1972; Arshavsky et al, 1984a). VSCT neurons seem to convey information on the central spinal activity during locomotion (Orsal et al, 1988).

Ventral spinocerebellar tract (VSCT) neurons receive afferent input from a number of proprioceptive and exteroceptive reflex pathways as well as descending systems involved in motor control

(Burke et al, 1971; Lundberg, 1971). After integrating and processing these inputs, the information is transmitted to the cerebellum by mossy fiber afferents (Bloedel & Courville, 1981; Lundberg, 1971; Oscarsson, 1973). These inputs include monosynaptic excitatory and disynaptic inhibitory input from Ia and Ib afferent fibers (Gustafsson & Lindstrom, 1973; Lindstrom & Schomburg 1973) and inputs from interneurons activated by flexor reflex afferents (Lundberg & Weight, 1971) Descending pathways modulating the excitability of VSCT cells include the rubrospinal (Baldissera & Bruggencate, 1976) and vestibulospinal (Baldissera & Roberts, 1975; 1976) systems. Based on the characteristics of the inputs from these different sources, Lundberg (1971) hypothesized that VSCT neurons act as comparators between the excitability change evoked in last-order inhibitory interneurons by spinal input and the excitability change in alpha motoneurons evoked by the same input. Lundberg's comparator hypothesis provided important insights into how the afferent input to alpha motoneurons may be processed by the VSCT. However, the possibility that the output of alpha motoneurons may modulate the excitability of this spinocerebellar pathway has not been thoroughly investigated.

Recent observations on the circuitry of the spinal cord have demonstrated collaterals of alpha motoneurons terminating in regions of the ventral horn which do not contain Renshaw cells (Cullheim et al, 1977). The possibility that collaterals of motoneurons can affect the excitability of inhibitory interneurons impinging on VSCT cells was addressed by Gustafsson and Lindstrom (Lindstrom & Schomburg, 1973). These investigators documented that ventral root stimulation can depress Ia IPSPs evoked in VSCT neurons by peripheral nerve stimulation. In a small fraction of VSCT cells, the activation of an inhibitory input to these cells by motoneuron axon collaterals has also been described (Gustafsson & Lindstrom, 1973; Lindstrom & Schomburg 1973). However, these studies did not elucidate any excitatory inputs to VSCT neurons activated by these collaterals. Only a depolarization presumably due to a disinhibitory mechanism was reported (Gustafsson & Lindstrom, 1973). Previous investigations also did not address whether the action of motoneuron collaterals can significantly influence the discharge of VSCT neurons. The purpose of this study was to demonstrate that motoneuron axon collaterals activate a short latency excitatory input to VSCT neurons and that this input has a marked effect on the discharge of these cells.

METHODS

Fifty adult cats (2.5~4.0 kg) were anesthetized with alpha-chloralose (60 mg/kg, i.p.). The right axillary artery and vein were catheterized to monitor blood pressure and to administer drugs, respectively. Tracheostomy and bilateral pneumothoraces were performed, and the animal was artificially respired. The animals were paralyzed with gallamine triethiodide. Expiratory CO2, body temperature and blood pressure were maintained within physiological ranges. Three concentric bipolar electrodes spaced 1.0 mm apart were placed in the right superior peduncle (see Fig. 1, S1). Two dorsal laminectomies were performed. A rostral laminectomy near the T6-T7 level permitted placement of a bipolar stimulating electrode on the cord contralateral to the side from which VSCT neurons were recorded (see Fig. 1, S2). A caudal laminectomy at the T10-S2 region exposed the lumbar enlargement of the spinal cord where VSCT neurons are concentrated(Burke et al, 1971; Ha & Liu, 1968; Hubbard & Oscarsson, 1962). After completion of the laminectomies a ventral root (L6 or L7) was isolated, transected slightly proximal to the dorsal root ganglion and mounted on a bipolar stimulating electrode(see Fig. 1, S3). An oil pool was formed from the skin flap and maintained at $38^{\circ} \pm 1^{\circ}C$ by a feedback controlled heating circuit.

VSCT neurons were recorded extracellularly with

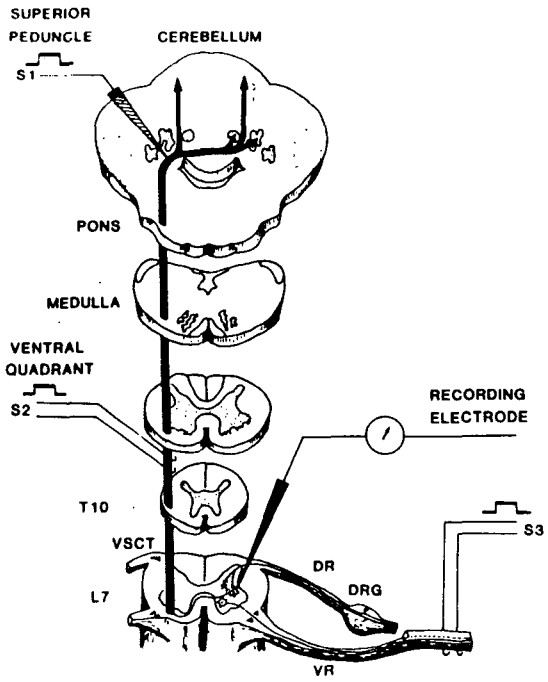

Fig. 1. A schematic drawing of the experimental design. The projection of VSCT is depicted as a heavy line. Abbreviations are as follow: DR, dorsal root; VR, ventral root; DRG, dorsal root ganglion.

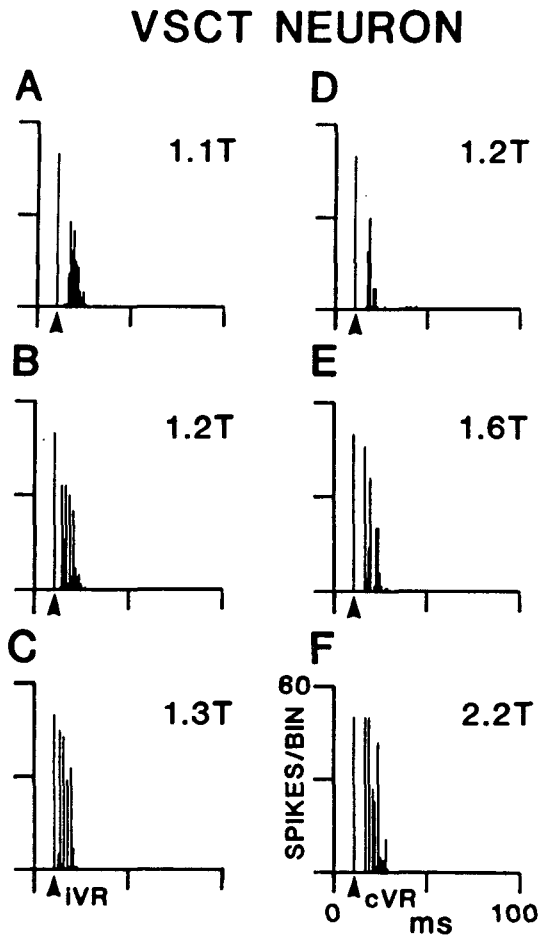

Fig. 2. PSTHs (S=50) of the response of a VSCT neuron to ipsilateral (iVR) and contralateral (cVR) ventral root stimulation. L7 ventral root was cut proximal to the L7 dorsal root ganglion, leaving L7 dorsal roots intact. T represents the threshold stimulus intensity required to just evoke the excitatory response. For this cell the threshold was 3.3V using a 0.2 msec stimulus duration. Responses are shown to stimuli of three different intensities applied to the ipsilateral ventral root (A,B,C) and the contralateral ventral root (D,E,F). Notice the increase in the amplitude of the response and a decrease in its latency as the stimulus intensity increased. Bin width = 1 msec. Solid arrow heads (A) in this and all subsequent figures indicate the time of the stimulus artifact.

glass microelectrodes filled with 2M sodium chloride (Z=2~3 Mega Ohm) using conventional techniques. A neuron was identified as a VSCT cell if it could be antidromically activated from the contralateral superior cerebellar peduncle. Criteria used for antidromic activation were: (1) the ability to follow high frequency stimulation (100~300 Hz) with a variation of less than 0.2 msec in latency; (2) change in latency of less than 0.2 msec when the stimulus intensity was increased from threshold to supramaximal amplitudes; and (3) collision of the antidromic spike with spontaneous activity. As a further requirement for identification, all VSCT neurons were checked for antidromic activation from the contralateral rostral cord. After successful identification of a VSCT neuron, its response to L6 or L7 ventral root stimulation was assessed by con-

structing a poststimulus time histogram (PSTH) of the discriminated unitary activity. Separate PSTHs were constructed to 100 consecutive ventral root stimuli with small gradation in intensity.

The intensity of the ventral root stimulus usually was determined by monitoring the amplitude of the antidromic field potential evoked in the ventral horn. Ventral root stimulus strength was expressed as a multiple of the intensity required to evoke the threshold response of the antidromic field. In a few experiments (Fig. 2) stimulus intensity was expressed as a multiple of that required to evoke the excitatory response to the VSCT cell. Stimuli with amplitudes of 2~8 volts and a duration of 0.1~0.2 msec were used. The stimulus artifact from the ventral root stimulus was usually large enough to result in a 2 msec period during which spike activity could not be determined adequately. Therefore, in many of the PSTHs the first 2 msec after the stimulus artifact were blank.

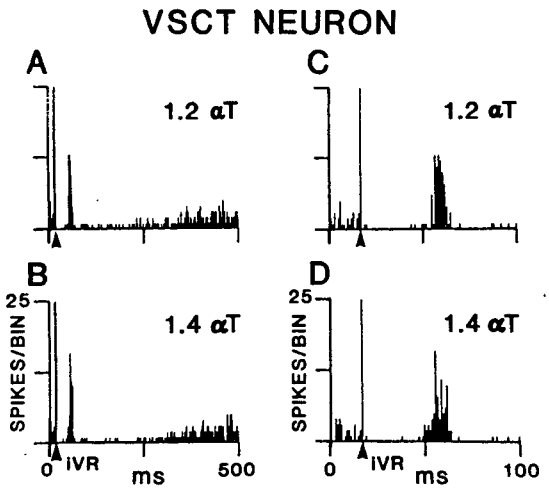

Fig. 3. PSTHs (S=100) of responses of a VSCT neuron to stimulation of the ipsilateral L7 ventral root. The threshold for evoking the responses are indicated in multiples of threshold for the activation of the antidromic alpha motoneuron field. Both a long latency excitatory response and long duration reduction of impulse activity was evoked in this cell. Responses are shown at two different sweep speeds in A-B and C-D.

RESULTS AND DISCUSSION

One hundred twenty eight VSCT neurons were antidromically activated with latencies between 3.0 and 8.0 msec following stimulation of the contralateral superior peduncle. Approximately 50% of the VSCT neurons were spontaneously active. Most neurons (n=104) responded to ipsilateral ventral root stimulation with either a short or long latency burst of action potentials (Table 1). The VSCT cell shown in Figure 2 responded to the ventral root stimulus with a short latency of 5~6 msec. Gradual increase in the intensity of ventral root stimulation (B and C) increased the amplitude of the response and decreased the latency to 2.0 msec at 1.3 times the threshold for evoking the excitatory response of this neuron. Contralateral ventral root stimulation also resulted in an increase in the impulse activity of this cell (D-F). The latency of the response to the contralateral root was slightly longer (7 msec) than the response to the ipsilateral stimulus at the lower

Table 1. Number of VSCT neurons activated by ipsilateral ventral root

	Number of cells	%
Activated	104	81.3
Not Activated	24	18.7
Total	128	100

stimulus intensity (D) and decreased to 6 msec at the higher stimulus intensity (F).

Longer latency excitatory responses to ventral root stimulation were also observed, as shown for the VSCT neuron in Fig. 3(also see Fig. 4). This excitatory response had a latency of 37 msec at stimulus intensities approximately 1.2 times the threshold for the alpha motoneuron field (A and C). The ventral root stimulus also resulted in a long lasting suppression of spike activity in many VSCT

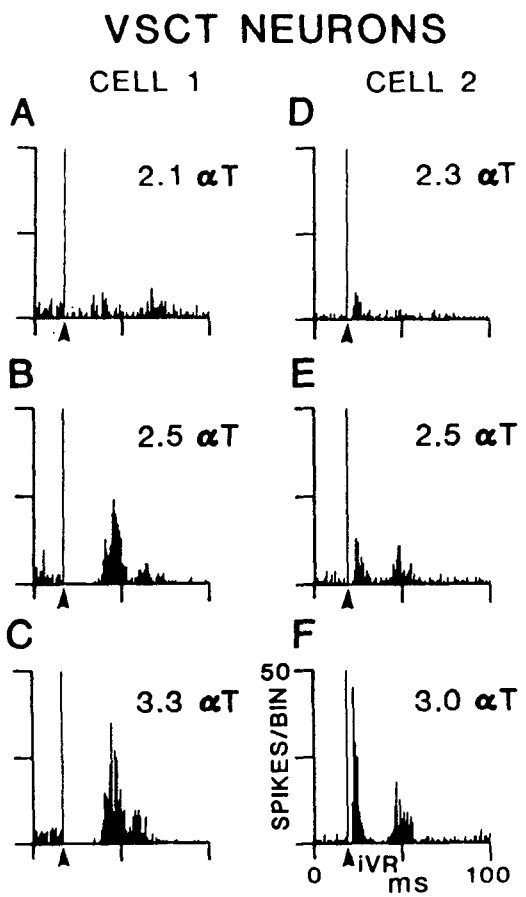

Fig. 4. PSTHs (S=100) of responses of two VSCT neurons to stimulation of the ipsilateral L7 ventral root. In CELL 1, only a long latency excitatory response(A-C) was evoked. On the other hand, in CELL 2, both a long latency excitatory response and long duration reduction of impulse activity was evoked (D-F). The threshold for evoking the responses are indicated in multiples of threshold for the activation of the antidromic alpha motoneuron field.

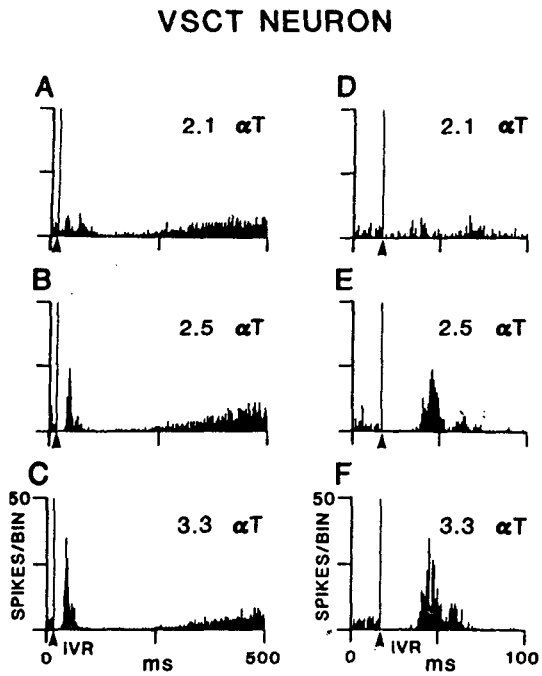

Fig. 5. PSTHs (S=100) of responses of a typical VSCT neuron to stimulation of the ipsilateral L7 ventral root, showing both excitatory and inhibitory responses. Note that responses are shown at two different sweep speeds in A-C and D-F.

neurons(Fig. 3 and Fig. 5). Because of the problem produced by the stimulus artifact described above, it was not possible to accurately estimate the latency of the decrease in the cell's discharge. However, the reduction of impulse activity had a latency as brief as 3~4 msec, and thus was evoked before the excitatory response. The duration of the decreased discharge rate was almost 300 msec (Fig. 3, A & B and Fig. 5, A-C). The threshold for the reduction in impulse activity was comparable to the threshold for the excitatory component of the response. Both the increase and the decrease in the cell's discharge were maximal at very low intensities. Increasing the stimulus intensity to 1.4 times alpha motoneuron threshold (Fig. 3, B & D and Fig. 5, B & C) had little additional effect on the response except for shortening the latency of the excitatory component. Although the thresholds of short latencies for the excitatory responses to ventral root stimulation suggest that these responses may not be evoked by the small diameter afferents in the ventral root (Clifton et al, 1974; Coggeshall, 1980; Coggeshall et al, 1980), additional experiments were carried out

to exclude this possibility. Ventral root afferents in the same segments were interrupted by carefully removing the dorsal root ganglion interrupting any fibers coursing from the ganglion to the ventral root. The fascicles of the ventral root were dissected and cut as far distally to the ganglionectomy as possible and mounted on bipolar stimulating electrodes(see Fig. 1, S3). The response characteristics of VSCT neurons to stimulating ventral root fibers in this preparation were identical to those recorded in preparations in which the ventral root was cut just distal to the dorsal root ganglion. In addition, responses in VSCT cells were evoked at stimulus intensities just above the threshold for activating the axons of alpha motoneurons.

To summarize the combined data from this study, 104 of 128 VSCT cells responded to stimulation of the ipsilateral ventral root with an increase in impulse activity (Table 1). A concomitant, prolonged decrease in impulse activity was observed in 23 of the 51 cells in which there was sufficient spontaneous discharge. In 37 cells in which the threshold for the antidromic alpha motoneuron field was monitored, the threshold for activation of the VSCT cells varied from 1.2 to 20 times threshold for the antidromic alpha motoneuron field. However, the majority of the neurons (26/37) were excited by ventral root volleys at less than 4.5 times alpha motoneuron threshold.

The results show that many VSCT neurons receive a strong excitatory input activated by motoneuron axon collaterals. These responses are not likely evoked by the small diameter afferents in the ventral roots root (Clifton et al, 1974; Coggeshall, 1980; Coggeshall et al, 1980). First, the shortest latency responses are too short to be evoked by these slowly conducting fibers in the ventral root (Clifton et al, 1974; Coggeshall, 1980; Coggeshall et al, 1980). Second, and most critical, these responses could be evoked by very low intensity stimuli. Furthermore, removal of the dorsal root ganglion in some studies did not change the characteristics of responses evoked by ventral root stimulation.

A comparison of the responses in Fig. 3, Fig. 4 and Fig. 5 with the depolarization responses observed in unanesthetized preparations by Gustafsson and Lindstrom (1973) suggests that the longer-latency responses observed in the present study could result from disinhibition of Ia inhibitory interneurons. These authors postulated an increase in the excitability of VSCT through a disynaptic pathway involving the inhibitory action of Renshaw cells on Ia inhibitory interneurons. However, it is unlikely that the short-latency excitatory responses observed here result from the same disinhibitory mechanism, since the time courses of these two responses are very different. Although segmental latencies of the disinhibition were as short as 2 msec Gustafsson and Lindstrom (1973), the response latencies shown graphically were 5~6 msec, the peak of the response occurred at 20 msec, and its duration was 40~50 msec. In the present study the response latency was often as short as 2 msec following stimulation of the ventral root just distal to the dorsal root ganglion. The latency to the peak of the response occurred very shortly after its onset, much shorter than the peak of the depolarization (20 msec) evoked by the disinhibitory mechanism (Gustafsson and Lindstrom (1973). Furthermore, the depolarization produced by the disinhibition of VSCT neurons evoked by ventral root stimulation was only observed in unanesthetized preparations Gustafsson and Lindstrom (1973), while the responses in this study were obtained in anesthetized animals. Therefore, it is most likely that the short latency responses reported here are evoked by the action of motoneuron collaterals on an excitatory input to VSCT neurons.

The long-duration reduction of spontaneous discharge observed in a number of VSCT cells may reflect the recurrent collateral inhibition described previously for a small number of VSCT neurons (Lindstrom & Schomburg, 1973). Eventhough

inhibition could be evaluated in only a small number of cells in this study due to the absence of spontaneous activity, the present data indicate that the inhibition following ventral root stimulation has a greater effect on the discharge of VSCT cells than could be inferred from the intracellular data (Lindstrom & Schomburg, 1973).

The observation that responses in some VSCT neurons could be evoked by stimuli just above threshold for the antidromic field of alpha motoneurons implicates the collaterals of these cells in evoking the response. However, other cells have response thresholds 2～5 times threshold for this field, suggesting that gamma motoneurons collaterals (Westbury, 1979) also may be involved. It is also possible, however, that these higher intensities were required to evoke a critical convergence of inputs from alpha motoneuron collaterals onto the interneurons involved in the pathway mediating the response. Additional studies are required to clarify this issue. The results in Fig. 2. also suggest that the activation of motoneuron collaterals can activate a crossed pathway modulating the excitability of contralateral VSCT neurons. This may relate to the observation that terminals of the motoneuron collaterals synapse on interneurons in the ventral horn projecting to ventromedial region of the contralateral cord (Oscarsson, 1973).

The relationship of these findings to the comparator hypothesis of Lundberg is not yet clear. This hypothesis focused on the capability of VSCT cells to compare the effects of afferent inputs to motoneurons with the effect of the same input on inhibitory interneurons. The present study demonstrates that the output of motoneuron collaterals also can produce a significant modulation of VSCT neuronal excitability. This effect of motoneuron output may contribute to the observation in the locomoting cat that dorsal root section did not affect the modulation of VSCT cells related to the locomotor cycle. More generally, the data indicate that the VSCT can provide the cerebellum with information concerning the output of the final component of the motor system.

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