Stimulation of a Restricted Region in the Midline Cerebellar White Matter Evokes Coordinated Quadrupedal Locomotion in the Decerebrate Cat

SHIGEMI MORI, TOSHIHIRO MATSUI, BUNYA KUZE, MITSURU ASANOME, KATSUMI NAKAJIMA, AND KIYOHJI MATSUAMAMA
Department of Biological Control System, National Institute for Physiological Sciences, Myodaiji, Okazaki 444-8585, Japan

Mori, Shigemi, Toshihiro Matsui, Bunya Kuze, Mitsuru Asanome, Katsumi Nakajima, and Kiyohji Matsuama. Stimulation of a restricted region in the midline cerebellar white matter evokes coordinated quadrupedal locomotion in the decerebrate cat. J. Neurophysiol. 82: 290–300, 1999. In the reflexively standing acute decerebrate cat, we have previously shown that pulse train microstimulation of the hook bundle of Russel in the midline of the cerebellar white matter, through which crossed fastigiofugal fibers descusate, augments the postural tone of neck, trunk, fore-, and hindlimb extensor muscles. In the present study we examined the possible role of such stimulation in evoking locomotion as the animal is supported by a rubber hammock with its feet contacting the moving surface of a treadmill. We were able to provoke well-coordinated, bilaterally symmetrical, fore- and hindlimb movements, whose cycle time and pattern were controlled by appropriate changes in stimulus intensity and treadmill speed. We carefully and systematically mapped this cerebellar locomotor region (CLR) through repeated dorsoventral penetrations with a glass-coated tungsten microelectrode in a single animal and between animals. We found that the optimal locus for evoking locomotion was centered on the midline, at Horsley-Clarke coordinates H0 and P7.0, and extended over a rostrocaudal and dorsolateral range of ~0.5 mm. The lowest effective stimulus intensity at the optimal site was in the range of 5–8 μA. Along penetration tracks to left or right of the midline, effective stimulus intensity increased and evoked locomotor patterns were no longer symmetrical, but rather shifted toward the contralateral limbs. In the same animals, controlled locomotion was evoked by stimulating the mesencephalic locomotor region (MLR). With concomitant stimulation of the optimal sites in the CLR and the MLR, each at subthreshold strength, locomotor movements identical to those seen with suprathreshold stimulation of each site alone were evoked. With concomitant stimulation at suprathreshold strength for each site, locomotion became vigorous, with a shortened cycle time. After making ablative lesions at either the CLR or MLR (unilateral or bilateral), controlled locomotion was still evoked at the prior stimulus strength by stimulating the remaining site. Together, these results demonstrate that selective stimulation of the hook bundle of Russel in the mid sagittal plane of the cerebellar white matter evokes “controlled” locomotion identical to that evoked by stimulating the MLR. We have shown that the fastigial nucleus is one of the supraspinal locomotion inducing sites and that it can independently and simultaneously trigger brain stem and spinal locomotor subprograms formerly believed to be the domain of various brain stem regions including the MLR and the subthalamic locomotor region.

INTRODUCTION

What is the role of the cerebellum and the cerebellar nuclei such as the fastigial nucleus in the control of posture and locomotion? Orlovsky (1970b) first demonstrated in decerebrated cats, whose nervous system was simplified by transection of the neuraxis at the thalamic or rostral mesencephalic level, that stimulation of the subthalmic locomotor region (SLR) in the lateral hypothalamic area and the mesencephalic locomotor region (MLR) in the posterior midbrain was still capable of evoking locomotor movements on the surface of a moving treadmill. In these animals, however, coordination of the fore- and hindlimbs was greatly disturbed, partly because of exaggerated straightening of the forelimbs due to very strong development of extensor rigidity. In the mesencephalic cat, the disturbances in coordination were more pronounced than those in the thalamic cat. These findings led to the general conclusion that the cerebellum is not directly involved in the initiation of locomotor movements but is concerned mainly with coordination of limb movements during ongoing locomotion (Armstrong 1978; Grillner 1981; Orlovsky and Shik 1976; Shik and Orlovsky 1976).

In humans and other mammals, it has been well established that midline cerebellar injuries and atrophies have, as their main consequence, instabilities of posture (trunkal ataxia) and locomotor movements (Dow and Moruzzi 1958). The cerebellar vermal cortex integrates proprioceptive, exteroceptive, visual, and vestibular afferent information that originates from a wide variety of sources (Armstrong 1978; Armstrong et al. 1997; Arshavsky et al. 1986). It is therefore likely that the vermis and the fastigial nucleus (to which efferent fibers from the vermis project) are concerned with maintaining posture and with elaborating associated body and neck movements, as suggested by Chambers and Sprague (1955a,b). Yu and Eidelberg (1983) demonstrated in cats that fastigial lesions, bilaterally, produced atactic locomotor movements. Because fastigioreticular, fastigiovestibular, and fastigiospinal pathways originate from the fastigial nucleus in addition to fastigiothalamic pathways (Asanuma et al. 1983a,b; Homma et al. 1995; Walberg et al. 1962a,b; Wilson et al. 1978), Armstrong (1986) proposed that the fastigial nucleus influences posture and locomotion by coordinating the activities of axial muscle groups via the reticulo- and vestibulospinal tracts. It has been well established that both the vestibulo- and reticulospinal fibers descend through the ventral and ventrolateral quadrants of the

The results of following stimulation and ablation studies supported the proposition that the reticulospinal pathway arising from the pontomedullary reticular formation (PMRF) is one of the major pathways mediating locomotor driving signals to the spinal cord, where the central pattern generator (CPG) for locomotion is located (Grillner 1975, 1981; Jordan 1991). In the decerebrate cat, Shik et al. (1968) stimulated the pyramidal tract at a site rostral to a bilateral transection and evoked locomotion in the same fashion as evoked by the MLR stimulation. Such stimulation was considered to activate collaterals of the pyramidal tract fibers, which presumably terminated in the PMRF. Studies in chronic adult cats (Afelt 1974; Eidelberg 1981) suggested that pathways in the ventral and ventrolateral quadrants are essential for the recovery of voluntary quadrupedal locomotion. Recent study by Brustein and Rossignol (1998) showed that the cats with a massive ventral and ventrolateral spinal lesion at low thoracic levels (T11-13) suffered from pronounced locomotor and postural deficits in the early period after the lesion, although all the cats eventually recovered quadrupedal voluntary locomotion possibly due to compensatory mechanisms.

In acute precollicular-postmammillary decerebrate cats maintaining a reflexively standing posture, we have recently reported that stimulation of a very restricted region of the cerebellar white matter along its midline resulted in augmentation of neck, lumbar back, fore-, and hindlimb extensor activities, bilaterally (Asanome et al. 1998; Mori et al. 1998a).

The effective stimulus region corresponded to the midline region of the hook bundle ofRussell (Rasmussen 1933), through which fastigioreticular, fastigiovestibular, and fastigiospinal fibers decussate (Brodal 1981; Homma et al. 1995; Matsushita and Iwahori 1971). We report here that stimulation of the same midline cerebellar region is capable as well of evoking locomotion in the mesencephalic cat on the surface of a moving treadmill. From the comparison of the characteristics of cerebellar and MLR-evoked locomotion in a single animal, we have suggested that locomotor driving signals arising from the fastigial nucleus and the MLR are relayed to the spinal cord, in part, by common reticulospinal pathways (Mori et al. 1998b). Results of the present study now support the proposition that the cerebellum is involved in the triggering of locomotor-related subprograms that reside within the brain stem and the spinal cord, and also shed considerable light on the functional role of the fastigial nucleus in the control of posture and locomotion in the cat. Preliminary results have been published elsewhere (Mori et al. 1998a,b).

**Methods**

Experiments were performed on 25 adult cats. Under halothane-nitrous oxide gas anesthesia, both carotid arteries were ligated. After surgical decerebration at the precollicular-postmammillary level, the head of the animal was fixed to a stereotaxic instrument together with dorsal spinal processes of the first three thoracic vertebrae. All wound surgical decerebration at the precollicular-postmammillary level, the nitrous oxide gas anesthesia, both carotid arteries were ligated. After

Electromyograms (EMGs) were recorded by implanting bipolar electrodes made of thin (100 μm) copper wires into selected extensor and flexor muscles of the left and right fore- (biceps brachii, BB; triceps brachii, TB) and hindlimbs (quadriceps femoris, QF; gastrocnemius-soleus, GS; tibialis anterior, TA). Recorded EMGs were displayed on a storage oscilloscope in various combinations and also stored on a digital tape recorder for later analysis. These EMGs were rectified and integrated when necessary.

Details of the systematic search of the cerebellar white matter for sites affecting locomotor behaviors have been published elsewhere (Asanome et al. 1998). Briefly, tungsten-in-glass microelectrodes with a tip diameter of 15–25 μm (impedance: 0.6–1.0 MΩ) were used for stimulation of the cerebellar white matter and the MLR. By means of independent micromanipulators, one electrode was introduced into the cerebellar white matter along its midline, and another into the left or the right MLR according to Horley-Clarke coordinates. In five animals, a third electrode was also inserted into the alternate right or left MLR. The first (cerebellar) electrode was advanced dorsoventrally in the cerebellar midline at steps of 0.1–0.25 mm through the depths from H + 2 to H – 2. Separate penetrations, at intervals of 0.1–0.25 mm, covered the A-P range from P6.5 to P7.5. Quasi-midline penetrations were made as well, from L2.0 to R2.0, and covered the same depth and rostrocaudal range as the midline (LR0) search. The second and the third electrodes were also inserted dorsoventrally into the MLR at 0.1- to 0.25-mm steps (P1.5 to 2.5, LR3.5 to 4.5, H + 1 to –1). The stimuli consisted of rectangular pulses of 0.2 ms duration in pulse trains of a frequency of 50 pulses/s. Stimulus intensity ranged from 5 μA to a maximum of 50 μA. In five experiments, the treadmill speed was changed in a stepwise manner over the range from 0.4 to 1.5 m/s while observing the effects of changes in the stimulus intensity delivered to the cerebellar locomotor region (CLR) and the MLR. The treadmill movement was routinely started before the CLR and MLR stimulation. In three experiments, stimulus frequency was increased from 20 to 300 Hz, and the relationships between stimulus frequency and stimulus threshold for evoking locomotion were studied.

During the experiments, the animal’s rectal temperature and mean blood pressure were monitored and kept at 36–38°C and >90 mmHg, respectively. End-tidal CO₂ was measured and maintained between 4 and 6%. Warmed physiological saline solution was frequently poured onto the exposed cerebellar surface. After identifying the stimulus sites within the midline cerebellar white matter, and the left and right MLR for evoking locomotion with the lowest stimulus intensity, small electrolytic lesions were made in a sequence by passing a DC cathodal current of 10 μA for 10 s. From the marked lesions, the optimal stimulus sites for evoking locomotion were identified. In a different group of animals (n = 5), large electrolytic lesions were made at the effective stimulus sites of the cerebellar white matter or the MLR. For this, a DC cathodal current of 30–50 μA was passed through the stimulating electrode for 30–40 s. These ablative lesions were made for the purpose of studying the effects of stimulating the intact CLR or unilateral/bilateral MLR while the counterpart was inactivated by destructive lesion.

At the end of each experiment, the cats were deeply anesthetized with pentobarbital sodium (40 mg/kg) and perfused transcardially for 20 min with 0.01 M phosphate-buffered saline followed by 4% Formalin solution. After the perfusion, both the cerebellum and brain stem were removed. After fixation in 10% Formalin solution, the sections were made in a systematic search of the cerebellar white matter along its midline, and another into the left or the right MLR according to Horsley-Clarke coordinates. In five animals, a third electrode was also inserted into the alternate right or left MLR. The first (cerebellar) electrode was advanced dorsoventrally in the cerebellar midline at steps of 0.1–0.25 mm through the depths from H + 2 to H – 2. Separate penetrations, at intervals of 0.1–0.25 mm, covered the A-P range from P6.5 to P7.5. Quasi-midline penetrations were made as well, from L2.0 to R2.0, and covered the same depth and rostrocaudal range as the midline (LR0) search. The second and the third electrodes were also inserted dorsoventrally into the MLR at 0.1- to 0.25-mm steps (P1.5 to 2.5, LR3.5 to 4.5, H + 1 to –1). The stimuli consisted of rectangular pulses of 0.2 ms duration in pulse trains of a frequency of 50 pulses/s. Stimulus intensity ranged from 5 μA to a maximum of 50 μA. In five experiments, the treadmill speed was changed in a stepwise manner over the range from 0.4 to 1.5 m/s while observing the effects of changes in the stimulus intensity delivered to the cerebellar locomotor region (CLR) and the MLR. The treadmill movement was routinely started before the CLR and MLR stimulation. In three experiments, stimulus frequency was increased from 20 to 300 Hz, and the relationships between stimulus frequency and stimulus threshold for evoking locomotion were studied.

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mediolateral, and rostrocaudal extent of ablative lesions, which were made within the cerebellar white matter and the MLR, was also estimated from the serial transverse sections of the cerebellum and the brain stem.

In five animals, a small volume of cholera-toxin b subunit conjugated peroxidase (CTb-HRP), which was dissolved in 0.01 M sodium phosphate buffer (0.4%, 50 nl, List Biological Laboratories), was focally injected into the lesioned site of the CLR by using a double-barreled pipette composed of a stimulating microelectrode and an injection glass pipette (Asanome et al. 1998; Ohta et al. 1988). The stimulating microelectrode was used for evoking locomotion as well as ablating the fibers of passage coursing through the lesioned site. For this purpose, a DC current of 10 μA was passed through this electrode for ~30 s. The attached pipette was used for microinjection of CTb-HRP solution into the lesioned stimulus sites. After survival periods of 12–48 h under continuous anesthesia with intravenous administration of physiological saline solution and warming of animals with a heating pad, the animals were deeply anesthetized and perfused transcardially with physiological saline solution followed by 4% Formalin solution.

In cats with CTb-HRP microinjection, the transverse 50-μm sections of the cerebellum and the brain stem were reacted for HRP-histochemistry using the tetramethyl benzidine (TMB) procedure (Mesulam 1982). All CTb-HRP-stained sections were examined under a light microscope equipped with a bright-field condenser, and the trajectory of fibers of passage was studied from serial sections as far caudally as possible. Photomicrographs were taken with a microscope (MICROPHOTO-FXA, NIKON, Tokyo, Japan) connected to a camera, and then scanned. To get an optimal reproduction of the staining, we modified the contrast and luminosity of the raw scan with Adobe Photoshop 4.0 on a Macintosh computer. The illustration was then printed with a digital color printer (Pictrography 3000, Fujifilm, Tokyo, Japan).

RESULTS

We inserted stimulating microelectrodes along dorsoventral tracks in the cerebellar midline or quasi-midline and were able to evoke locomotion on the surface of the moving treadmill with coordinated fore- and hindlimb movements in all animals. At optimal sites along the appropriately placed tracks, we could elicit such movement with stimulus intensity as low as 5 μA. At such sites, the maximum effective stimulus intensity decreased sharply with increase of frequency over the range of 20–50 Hz, and then remained almost constant over the range of 50–300 Hz. We limited test stimulus intensity to a maximum of 50 μA at all sites.

The first experiments consisted of a systematic mapping of the sites within the cerebellar white matter from which such locomotor behavior could be elicited. We also studied the trajectory of fibers passing through these locomotor regions by using the CTb-HRP tracing technique. We then investigated the possibility of controlling the pattern of the CLR-evoked locomotion with systematic changes of stimulus intensity and treadmill speed. And finally, we sought to clarify the relationship of cerebellar-induced locomotor mechanisms to those of other systems relaying through the PMRF such as the MLR.

Rostrocaudal, dorsoventral, and mediolateral extent of the effective cerebellar regions for evoking locomotion

We first searched out the rostrocaudal and dorsoventral extent of the optimal region for evoking locomotion in the cerebellar midline. Figure 1 shows responses to stimulation along three electrode tracks in one animal. The loci of optimal stimulus sites along tracks a, b, and c (at P6.85, P7.1, and P7.35, respectively) are plotted on the midsagittal (left) and frontal (right) planes of the cerebellum in Fig. 1A. We found all effective sites to be rostral to or just adjacent to the rostral pole of the fastigial nucleus (FN). Along the most rostral and caudal tracks, locomotor behavior occurred only with stimuli at points a and c (with intensities of 30 and 20 μA, respectively). The vertical bar through point b represents the range over which stimuli were effective. The points a and c, along with the bar through b, trace out an

![FIG. 1. Stimulus sites of the cerebellar white matter (A) and electromyograms (EMGs) recorded from fore- and hindlimb muscles during locomotion (B). A: plots of the locations of 3 stimulus loci (a–c) on the midsagittal plane (left) and of the same sites projected onto the frontal plane at P7.0 (right). Axes show Horsley-Clarke coordinates. FN, fastigial nucleus; INA, interpositus nucleus, anterior division; IV, fourth ventricle; VLD, dorsal portion of the lateral vestibular nucleus. B: EMGs recorded during locomotion evoked by stimulating the 3 sites (a–c) in A. L, left; R, right; BB, biceps brachii; TB, triceps brachii; TA, tibialis anterior; GS, gastrocnemius-soleus muscles. Horizontal bar beneath the bottom trace represents 2 s.](http://jn.physiology.org/Downloaded-from-by-10.220.33.5-on-June-21,2017)
evoked locomotion with stimulus intensities of 20–25 mA, the animal extended the forelimbs with an increased activity of the TB muscles, bilaterally, and exhibited hindlimb walking with alternating bursting discharges of TA and GS muscles. Mean interburst interval of these muscles was 0.77 s. During stimulation at site c with the lowest effective stimulus intensity of 20 μA, the animal flexed the hindlimbs, with an increased activity of the TA muscles, bilaterally, and now exhibited forelimb walking with alternating bursting discharges of BB and TB muscles. Mean interburst interval of these muscles was 0.53 s. By stimulating regions located further rostrally or caudally to the stimulus sites a and c, no locomotor movements were evoked even with stimulus intensity as high as 50 μA.

By stimulating site b, which corresponded to the dorsoventral depth of H = 0.3, we evoked full locomotion with a cycle time of 0.73 s at the lowest stimulus intensity of 15 μA. Along this track, we could also evoke locomotion by stimulating the sites at H + 0.1 and H + 0.5, but now with the minimum stimulus intensity of 20 μA. By stimulating the regions located further dorsally or ventrally to that marked by the vertical bar on the midsagittal plane in Fig. 1A, left, we could no longer evoke postural changes and/or locomotor movements with the stimulus intensity as high as 50 μA. By stimulating optimal sites at the depth of H + 0.2 to H + 0.4 along the P7.0 and P7.2 midline tracks, we evoked locomotion with stimulus intensities of 20–25 μA. Although the stimulation delivered to the best loci provoked fore- and hindlimb coordinated locomotion, stimulation of sites located rostrally or caudally from the best loci occasionally provoked locomotion with slightly different frequencies of fore- and hindlimb movements. In this animal, we did not further systematic stimulation study because electrolytic lesions were made at each of the stimulus sites a, b, and c.

The two photomicrographs in Fig. 2 were taken from the midsagittal and frontal sections of the cerebellum and the brain stem from two animals. The photomicrograph in Fig. 2A shows three stimulus tracks and a single lesion site from the animal shown in Fig. 1A, left. The leftmost (most rostral) track was made as a reference track. Along the next track as indicated by a thin vertical arrow, the microlesion of a site a is marked by the thick arrow. The rightmost track is the one on which the stimulus site b is located. The microlesion at site b and the stimulus track along the stimulus site c were not clearly seen on this midsagittal section. The photomicrograph in Fig. 2B illustrates the microlesion (thick arrow) that was made at the most effective stimulus site (P7.0, LR0, H0) in the animal shown in Fig. 3A. The position of the cerebellum was slightly distorted in relation to that of the brain stem. The effective stimulus site for evoking full locomotion was located within the fiber bundle dorsal to the most ventral part of the gray matter in the midline of the cerebellum.

In the animal in Fig. 3A, the dorsoventral extent of effective stimulus sites along the midline of the cerebellum was studied. The stimulus track was located at the P7.0 level. Stimulus intensity was fixed to 15 μA. By stimulating three sites along the midline at H + 0.25 (a), H0 (b), and H-0.25 (c), full locomotion was provoked with initial rhythmic movements of the hindlimbs. The latency, which was measured from the onset of cerebellar stimulation to the beginning of the first bursting discharge of GS muscles, was as short as 1.0 s for the locomotion evoked by stimulating the site at H0 (b). The cycle time of locomotion was ~0.70 s. By stimulating the site at H0, full locomotion was still evoked even when the stimulus intensity was decreased to 8 μA, but the cycle time was prolonged to 0.90 s. Stimulation at H + 0.25 (a) or H-0.25 (c), with the same intensity of 8 μA, failed to evoke locomotion. Stimulation of sites at H + 0.1 and H - 0.1 with minimum intensity of 10–12 μA evoked locomotion. With the stimulus delivered to more dorsally or ventrally located midline regions above H + 0.25 or below H - 0.25, we could not evoke locomotor movement even with the highest stimulus intensity of 50 μA.

In a different animal, we stimulated sites on different sides of the midline (Fig. 3B). We inserted electrodes directed at the rostral pole of the fastigial nucleus at L2.0 and R2.0, as well as LR0 at the rostrocaudal level between P7.0 and P7.3. The stimulus intensity applied along each track was fixed at 20 μA. Three sets of EMGs in Fig. 3B were recorded from the left and the right forelimb muscles. In this animal, stimulation of the midline region at the depth of H + 0.25 at P7.0 level provoked full locomotion with the lowest stimulus intensity of 8 μA, and with cycle time of 0.46 s (b). During the midline-evoked locomotion, the BB and TB muscles exhibited regular and symmetrical alternating bursting discharges.
Postmortem study of histological sections showed that the most effective stimulus sites targeting LR0 and R2 were located along the midline and 2 mm lateral from the midline of the cerebellum, but the site targeting R2.0 was located at R2.3. The stimulus sites along the lateral tracks were located adjacent to the most rostral and dorsal part of the left and the right fastigial nuclei, respectively. With stimulation of the optimal site located at L2.0, H-0.2, the animal walked with marked deviation of the right fore- and hindlimbs to the right-hand side. Bursting discharges of the left BB and TB muscles were remarkably diminished while those of the right BB and TB muscles were augmented (a). The left fore- and hindlimbs also deviated to the right-hand side as if the animal was trying to turn or to walk diagonally on the treadmill. With stimulation of the optimal site located at R2.3, H-0.2, locomotor movements with a pattern opposite to those evoked by stimulating L2.0 region were evoked. Now the animal walked with marked deviation of the left and the right fore- and hindlimbs to the left-hand side. The bursts of activity in the left and the right forelimb muscles were augmented and diminished, respectively (c). The cycle time of the locomotion evoked by stimulating the two sites at L2.0 and R2.3 was ~0.57 and 0.58 s, respectively. By decreasing the stimulus intensity from 20 to 15 μA at these lateral sites, the degree of body tilting was slightly diminished, but asymmetry of the locomotor movements persisted. The cycle time of the locomotion was also prolonged by 0.1–0.2 s.

In this animal, the dorsoventral extent of the effective regions along the midline track was ~0.5 mm as in the first animal illustrated in Fig. 1. In contrast, the dorsoventral extent of effective regions along the laterally located tracks increased to ~0.6–0.8 mm. Along the lateral tracks, optimal stimulus sites were located slightly ventral to those in the midline. Locomotion evoked by stimulating the sites located along the lateral tracks always evoked asymmetric movements of the fore- and the hindlimbs, and the closer the lateral stimulus sites were to the midline, the smaller was the asymmetry of the evoked locomotion. These results suggest that locomotor movements can be evoked by stimulating a barrel-shaped neural region, which lies transversely within the cerebellar white matter between the rostral poles of the left and right fastigial nuclei.

**Representative location of a CTb-HRP–injected microlesion and trajectory of CTb-HRP–labeled fibers**

By focally injecting CTb-HRP in the lesioned effective site for evoking locomotion, it was possible to trace the anterogradely labeled fibers that passed through the lesioned sites. Across animals with different survival periods up to 48 h, we obtained essentially the same results. The photomicrograph in Fig. 4A illustrates the lesioned site on the frontal plane at P7.1. The shape of the microlesion was oval with a diameter of ~0.3 mm, and it was surrounded by a laterally extended diffusion area of CTb-HRP. The two photomontages in Fig. 4, B and C, were made from the photomicrographs, which were taken from the sections slightly rostral to the CTb-HRP injection site. CTb-HRP–labeled fibers appeared as densely stained, rod-shaped structures of various calibers. The length of rods was shorter at the quasi-midline region and longer at the lateral region. A small portion of these fibers passed through the rostral portion of the left and the right fastigial nuclei. These findings indicate that the fibers run rostrally from the CTb-HRP injection site and then transversely at the lateral region.

The photomontages in Fig. 4, B and C, show a bundle of labeled fibers running transversely to the left and to the right. The labeled fibers curved along the dorsal part of the left and the right brachium conjunctivum (BC), respectively. These fibers then descended ventrally along the lateral border of superior and lateral vestibular nuclei. Some of them continued to run to the PMRF and the vestibular complex. The trajectory of the CTb-HRP–labeled fibers was essentially the same as that identified by focal microinjection of an anterograde neural tracer, Phaseolus vulgaris leucoagglutinin, into the fastigial nuclei (Homma et al. 1995). These results together with demonstration of the selective location of the retrogradely labeled cells in the fastigial nuclei (Asanome et al. 1998; Mori et al. 1998a) demonstrated that the cerebellar-evoked locomo-
tion was due to activation of the hook bundle of Russel, through which crossed fastigiofugal fibers decussate.

Comparison of CLR- and MLR-evoked locomotion, and summation effects of the CLR and MLR stimulations

To elucidate the characteristics of CLR-evoked locomotion, effects of CLR and MLR stimulation were first compared. Stimulation of the CLR (P7.0, LR0, H + 0.2) for 5 s with the stimulus intensity of 10 μA and with the treadmill speed of 0.6 m/s evoked coordinated locomotion with latency of ~1.5 s after the stimulus onset as shown in the EMG records in Fig. 5A. Cycle time was ~0.70 s. After termination of the stimulation, locomotion continued for ~8–10 steps with a gradual prolongation of cycle time and weakening of the bursting discharges of the left and the right TA and GS muscles. Stimulation of the MLR in the same animal (P2.0, L4.0, H0) with the same parameters of stimulus intensity and duration evoked locomotion with latency of ~1.5 s and with cycle time of ~0.68 s (Fig. 5B). The CLR- and MLR-evoked locomotion were qualitatively indistinguishable.

We also found that concomitant stimulation of CLR and MLR, at subthreshold intensity for each locus, evoked locomotion like that evoked by separate suprathreshold stimulation (not illustrated). Integrated EMGs made from the records obtained during separate and simultaneous CLR and MLR stimulation are illustrated in Fig. 6. The stimulus site in the CLR was at P6.9, LR0, H + 0.4, and that in the MLR was at P2.0, L4.2, H0. The treadmill speed was kept constant at 0.8 m/s. MLR stimulation alone at stimulus intensity of 15 μA evoked locomotion with cycle time of ~0.60 s (Fig. 6A). CLR stimulation alone at stimulus intensity of 10 μA also evoked locomotion with cycle time of 0.60 s (Fig. 6B). When these stimulations were combined, locomotion became vigorous. Individual bursting discharges of both the TB and GS muscles became stronger in both amplitude and duration compared with those observed during the locomotion evoked by either the MLR or the CLR alone.

To further evaluate the characteristics of CLR-evoked locomotion, we systematically changed the treadmill speed and the stimulus intensity, and studied the changes in the patterns of evoked locomotion. We then examined the patterns of MLR-evoked locomotion in a similar manner in the same animal. The results are summarized in Fig. 7. In this animal, stimulus sites of the CLR and the MLR were located at P7.0, LR0, H + 0.2, and at P2.0, L4.2, H0, respectively. EMGs in Fig. 7, A and B, were recorded during CLR-evoked locomotion in relation to the stepwise changes in the treadmill speed and the stimulus intensity, respectively. We first evoked full locomotion by stimulating the CLR with constant stimulus intensity of 10 μA. With an increase in the treadmill speed from 0.7 m/s (a) to 1.1 m/s (b) and then to 1.4 m/s (c), the duration of bursting periods of TB and GS muscles, bilaterally, decreased remarkably with...
We assessed the degree of ablative lesions by stimulating theobilaterally, obliterating completely the effective lesion.

Effects of ablative lesions at either the CLR or MLR

Finally, we made ablative lesions in either the bilateral MLR or the CLR, and we studied effects of stimulating the counterpart. This study was aimed at answering the question of whether or not the CLR and the MLR are functionally independent locomotion inducing loci or mutually dependent loci for evoking locomotion. We held the location of the stimulating electrode fixed once the optimal stimulus site was identified. Observations and EMG recordings were made 5–10 min after making ablative lesions in the locomotion inducing sites. We assessed the degree of ablative lesions by stimulating the lesioned sites with the stimulus intensity >100 µA, which was 5–10 times stronger than the suprathreshold stimulus intensity for evoking locomotion in the same sites before making the lesions. With this stimulus intensity, no sign of locomotor movements was noted.

We found histologically identified lesions in the cerebellar white matter to extend ~0.5 mm, dorsoventrally, rostrocaudally, and mediolaterally, obliterating completely the effective stimulus loci for evoking locomotion. The area of the lesions made in the left and the right MLR also ablated those stimulus loci from which locomotion could usually be evoked. After making lesions, locomotion was evoked on stimulation of the remaining intact site with patterns similar to those before making the lesions. The stimulus intensity needed for evoking locomotion was similar to that before making ablative lesions in the MLR, unilaterally and bilaterally. Essentially similar results were obtained by stimulating the MLR after making ablative lesions in the CLR. These results demonstrated that the CLR and the MLR are independent locomotion inducing sites and are capable of activating brain stem–spinal locomotor subprograms commonly and separately.

**DISCUSSION**

The results of the present study demonstrated clearly that stimulation of the hook bundle in the midline of the cerebellum is capable of evoking coordinated locomotion on the surface of a moving treadmill, suggesting that fastigial cells are capable of initiating and controlling locomotion in the mesencephalic cat.

*Locomotion inducing sites and functional role of the reticulospinal cells in the initiation of locomotion*

In the mesencephalic cat, Shik et al. (1966) first demonstrated that MLR stimulation evokes “controlled” locomotion on the surface of a moving treadmill. The MLR was considered to correspond to the caudal portion of the cuneiform nucleus. In the same preparation, Shik et al. (1968) also showed that stimulation of the rostral pyramidal tract provoked locomotion provided that the pyramidal tracts were sectioned bilaterally at the medullary level caudal to the pyramidal stimulus sites. Such stimulation evoked only walking or trotting, and the pyramidal tract–evoked locomotion was in general much less stable than MLR-evoked locomotion. With concomitant stimulation of the MLR and the pyramid, each at subthreshold strength, locomotion was also evoked. In the pyramidal tract–sectioned cat, rostral pyramidal stimulation still evoked locomotion even after making ablative lesions in the MLR, bilaterally. Shik et al. (1968) suggested that pyramidal tract–evoked locomotion was due to an activation of reticulospinal cells in the PMRF by way of corticoreticular fibers. Clear evidence for an anatomic basis for this proposition was recently provided by Matsuyama and Drew (1997). MLR-evoked locomotion was also demonstrated to be due to an activation of reticulospinal cells (Garcia-Rill and Skinner 1987; Orlovsky 1970b,c; Shik and Orlovsky 1976).

The physiologically identified MLR was found, anatomically, to project to the magnocellular (FTM) and gigantocellular (FTG) tegmental fields of the medullary reticular formation (MRF) with ipsilateral dominance (Garcia-Rill et al. 1983;
Intracellular (Orlovsky 1970c) and extracellular recording studies (Garcia-Rill and Skinner 1987) also showed that MLR stimulation results in mono- and polysynaptic activation of reticulospinal cells in the PMRF. In addition to the MLR, Garcia-Rill (1991) suggested that the pedunculopontine nucleus (PPN) is also one of the locomotion-inducing site in the midbrain. They found that efferent fibers from the PPN/MLR projects to the transition zone between FTM and FTG of the MRF. These results suggested that the major pathways mediating the stimulus effects of the MLR/PPN complex is through the FTM, especially the reticulospinal pathways that originated from the nucleus reticularis magnocellularis (NRMc) (Garcia-Rill et al. 1983; Jordan 1991).

Cerebellar and brain stem neuronal mechanisms related to initiation and control of posture and locomotion

During MLR-evoked locomotion of the decerebrate cats on the moving treadmill, Orlovsky (1970a) first recorded the activity of reticulospinal cells in the FTG and found that these cells exhibit, besides tonic activity, rhythmical discharges. In a similar locomotor preparation, Orlovsky (1972) also found rhythmically discharging fastigial cells with tonic activity. The frequency curve for the “average” fastigial neuron showed maximum activity approximately in the swing phase of the ipsilateral hindlimb and in phase with that of the reticulospinal cells. These observations demonstrated that fastigial and reticulospinal cells are in fact involved in the control of MLR-evoked locomotion. The finding that fastigial cells exhibited rhythmical discharges during the period of the pinna-evoked fictitious scratch reflex in immobilized cat (Antziferova et al. 1980) suggests that some of the rhythmical fastigial cell discharges might be centrally generated, as opposed to being due to peripheral feedback from the moving limb.

To answer the question of whether or not the fastigial nucleus is mainly involved in the modulation of locomotion or whether it is also capable of initiating locomotion or both, it is necessary to understand the function of reticulospinal cells. Ito et al. (1970) and Eccles et al. (1975) demonstrated that stimulation in or near the fastigial nucleus resulted in orthodromic activation of reticulospinal cells in the FTG. The fastigial nucleus seems to have the potential capability of initiating locomotion by activating reticulospinal cells. However, highly complex effects on posture and locomotion were evoked by stimulating the fastigial nucleus (Batini and Pompeiano 1958; Moruzzi and Pompeiano 1956). Such complex effects can be the result of concomitant activation of corticofastigial and corticovestibular Purkinje cell axons, together with cells of...
origin of fastigiofugal fibers and certain cerebellar afferent fibers (Mori et al. 1998a). Before further discussing the fastigial contribution to the initiation and control of locomotion, it will be necessary to understand the specific trajectory of fastigiofugal fibers in relation to the hook bundle and the CLR.

**Trajectory of crossed fastigiofugal fibers passing through the CLR and their termination areas in the PMRF**

Rasmussen (1933) first demonstrated that both crossed and uncrossed fastigiofugal fibers originate from the fastigial nucleus. Approximately one-half of the fastigiofugal fibers were found to decussate, pass through the rostral part of, and rostral to the opposite fastigial nucleus to form the hook bundle of Russell before entering the cerebellar peduncle. These fibers converged as they approach the midline region of the cerebellar white matter (Matsushita and Iwahori 1971). Uncrossed fibers coursed caudally medial to the brachium conjunctivum and then ventrolaterally to the vestibular complex. Crossed fastigiofugal fibers include fastigioreticular, fastigiovestibular, and fastigiospinal fibers terminating at the upper cervical cord (Fukushima et al. 1977; Walberg et al. 1962a,b). Fastigiofugal fibers, which originated from the rostral, middle, and caudal parts of the fastigial nucleus, tended to pass through the lower, middle, and upper portion of the hook bundle, respectively. Each of these fiber groups terminated in the specific regions of the FTG (Homma et al. 1995).

In the mesencephalic cat placed on a still surface, we showed that midline stimulation of the hook bundle, with the stimulus intensity subthreshold for evoking locomotion, provoked general augmentation of postural muscle tone (Asanome et al. 1998; Mori et al. 1998a). By increasing the stimulus intensity, we found that the same animal exhibits locomotor-like movements on both the stationary and moving treadmill surface. Before the initiation of locomotion, the level of postural muscle tone was routinely increased. By a CTb-HRP retrograde tracing method, we found that cells of origin of fibers that projected to the CLR were located exclusively in the fastigial nuclei, bilaterally (Asanome et al. 1998). The trajectory of descending fibers coursing through the CLR was found in this study to be essentially the same as that identified by injecting an anterograde neural tracer, *Phaseolus vulgaris* lectin-coagglutinin, into the fastigial nucleus (Homma et al. 1995). These results disclose clearly that the CLR corresponds to the hook bundle in the midline of the cerebellum, and that CLR stimulation exerts dual effects on posture and locomotion.

**Effects of CLR stimulation on reticulospinal cells and on induced locomotion**

The characteristic trajectory of fastigiofugal fibers indicates that stimulation of the hook bundle results in a selective and simultaneous activation of crossed fastigioreticular, fastigiovestibular, and fastigiospinal fibers, descending bilaterally to the brain stem and the spinal cord. Midline stimulation would activate decussating fastigial fibers and in turn activate both the left and right PMRF to a similar extent (Mori et al. 1998a). An increase in the stimulus intensity would result in an increase in the number of excited reticulospinal cells, and in consequence enhance locomotor driving signals descending to the spinal cord. In contrast, stimulation of the hook bundle at a locus located laterally to the midline would result in a predominantly unilateral activation of fastigiofugal fibers before decussation of the fibers at the midline, resulting in recruitment of a larger number of reticulospinal and vestibulospinal cells located contralaterally to the stimulus site.

We evoked widely varying patterns of locomotion, depending on the locus of stimulation. Stimulus site–specific differences in the evoked locomotor patterns may be related to the differences in the activated group of fastigiofugal fibers within the hook bundle, and to termination sites of fastigioreticular and fastigiovestibular fibers in the PMRF (Homma et al. 1995). Based on the results obtained by microstimulation in the intact unanesthetized cat, Drew and Rossignol (1990a,b) suggested that the MRF is topographically organized, but without usual precision of such organization. Movements of the head were obtained from the whole extent of the brain stem. Ipsilateral forelimb movements were preferentially evoked by stimulation to the dorsal MRF, whereas contralateral forelimb movements and movements of the hindlimbs were evoked from more ventral and rostral regions. At present, however, it is not possible to directly correlate various patterns of CLR-evoked locomotion to activated components of the fastigioreticular and/or fastigiovestibular fibers, and topographical organization of the PMRF.

We have recently found that reticulospinal cells in the NRGc were monosynaptically activated by CLR stimulation (Matsui et al. 1997b; Mori et al. 1998b). Some of these cells were also activated, mainly polysynaptically with MLR stimulation. The NRGc reticulospinal cells, which projected to the lumbar segments, increased their discharge frequency remarkably during CLR-evoked locomotion with some phasic modulation (Matsui et al. 1997b; Mori et al. 1998b). The conduction velocity of these NRGc cells was faster than 55 m/s. The maximum activity of these cells was found at diverse times of the step cycle in the hindlimbs, as in the study of Drew et al. (1986) in intact cats walking on the treadmill. During the CLR-evoked tonic discharges of these NRGc cells, we found individual spikes that were locked to each stimulus pulse of the CLR stimulation. This result suggested that CLR-evoked locomotor driving signals to the spinal cord were relayed by the NRGc reticulospinal cells. Such a proposition, however, does not rule out the possibility that some of them are mediated by the NRMc reticulospinal cells in parallel, because a part of the fastigioreticular fibers terminated in the FTM (Homma et al. 1995).

**Control of locomotion by cerebellar nuclei and the cerebellum**

Major functions of the cerebellum have been considered to be integration of multi-modal afferent information, storage of motor programs, motor learning, and triggering of the motor programs (Bloedel 1992, 1995; Brooks and Thach 1981; Houk et al. 1993; Ito 1984). The fastigial nucleus is located ideally at a key station of a functional spinocerebellar “closed loop” formed between the neural elements in the cerebellum, brain stem and spinal cord (Armstrong 1986; Arshavsky et al. 1986). Because execution of proper locomotion requires simultaneous control of head, body, and limb movements in addition to balance control, fastigiospinal, fastigioreticular, and fastigiovestibular fibers can be considered to carry command signals...
related to head-neck movements (Wilson et al. 1978), activation of the CPG (Grillner 1981; Jordan 1991), and the maintenance of balance. Our preliminary study showed that some of the vestibulospinal cells were monosynaptically activated by CLR stimulation (Matsui et al. 1997a). This study was supported by Ministry of Education, Science, Sports and Culture of Japan Grant in Aid for Scientific Research A06404087 to S. Mori.

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