Coupling Between Feline Cerebellum (Fastigial Neurons) and Motoneurons Innervating Hindlimb Muscles

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Matsuyama, Kiyoji and Elzbieta Jankowska. Coupling between feline cerebellum (fastigial neurons) and motoneurons innervating hindlimb muscles. J Neurophysiol 91: 1183–1192, 2004; 10.1152/jn.00896.2003. The aims of the study were twofold: (1) to verify the hypothesis that neurons in the fastigial nucleus excite and inhibit hindlimb α-motoneurons and (2) to determine both the supraspinal and spinal relays of these actions. Axons of fastigial neurons were stimulated at the level of their decussation in the cerebellum, within the hook bundle of Russell, in deeply anesthetized cats with only the right side of the spinal cord intact. The resulting excitatory postsynaptic potentials and inhibitory postsynaptic potentials were analyzed in motoneurons on the left side of the lumbar enlargement. Postsynaptic potentials evoked by the first effective stimulus were analyzed in motoneurons on the left side of the lumbar enlargement. Postsynaptic potentials evoked by the first effective stimulus were analyzed in motoneurons on the left side of the lumbar enlargement. Postsynaptic potentials evoked by the first effective stimulus were analyzed in motoneurons on the left side of the lumbar enlargement. Postsynaptic potentials evoked by the first effective stimulus were analyzed in motoneurons on the left side of the lumbar enlargement.

INTRODUCTION

Neurons in the fastigial nucleus are of prime importance in the control of movements of the eyes and of the head (for reviews see, e.g., Gamlin 1999; Wilson et al. 1978). However, they may also influence postural and locomotor reactions of the whole body (Mori et al. 1998, 1999). Early observations on effects of stimulation of the fastigial nucleus on hindlimb motoneurons suggested that these effects were evoked via polysynaptic pathways, in view of the long (10–20 ms) latency of the earliest excitatory postsynaptic potentials (EPSPs) (Sasaki and Tanaka 1963) and of long duration of facilitation of monosynaptic reflexes (Asratian and Grigorian 1979). However, subsequent studies indicated that the earliest fastigial actions could be evoked by much more direct pathways. For instance, fastigial actions could be evoked di- or trisynaptically if they were mediated by reticulospinal, vestibulospinal, or long propriospinal neurons that are monosynaptically activated by fastigial neurons (Alstermark et al. 1987a,b; Homma et al. 1995; Mori et al. 1998) and have mono- or disynaptic actions on hindlimb motoneurons. Coupling between long propriospinal neurons and motoneurons has not yet been investigated but mono- and disynaptic coupling between reticulospinal or vestibulospinal neurons and ipsilateral α-motoneurons has been long known (Floeter et al. 1993; Gossard et al. 1996; Grillner and Lund 1968; Jankowska et al. 2003; Lund and Pompeiano 1968; Shapovalov 1969; Wilson and Yoshida 1969). Disynaptic coupling from both reticulospinal and vestibulospinal neurons has been also found for contralateral α-motoneurons (Aoyama et al. 1971; Bannatyne et al. 2003a,b; Gossard et al. 1996; Grillner 1970, 1971; Hongo et al. 1971; Jankowska et al. 2003; Krutki et al. 2003; Maeda et al. 1975; Shapovalov 1969).

The main aim of this study was therefore to verify the existence of trisynaptic coupling between fastigial neurons and contralateral α-motoneurons via commissural interneurons, as diagrammatically indicated in Fig. 1A. The second aim was to investigate whether any EPSPs or inhibitory postsynaptic potentials (IPSPs) evoked in this way were mediated via reticulospinal or vestibulospinal tract fibers or by both. This was done by establishing whether spatial or temporal facilitation occurs at a premotoneuronal level between effects of stimuli applied to axons of fastigial neurons (within the hook bundle of Russell) (Rasmussen 1933) and to axons of reticulospinal neurons within the medial longitudinal fascicle (MLF) in the medulla or within the lateral vestibular nucleus (LVN). Since MLF contains reticulospinal tract fibers originating either in pontine or in bulbar reticular nuclei (Basbaum et al. 1978; Matsuyma et al. 1988 1993; Mitani et al. 1988a), no attempt was made to differentiate between effects of reticulospinal fibers of different origin. Results of pilot experiments (Nakajima et al. 2000) and preliminary results of this study (Matsuyama and Jankowska 2003) have been published in an abstract.

METHODS

Preparation

The experiments were performed on seven deeply anesthetized cats (weighing 2.6–3 kg). Anesthesia was induced by pentobarbital sodium (40 mg/kg ip) and maintained first with pentobarbital (in doses of 1–2 mg/kg iv up to a total of 45 mg/kg), and thereafter with α-chloralose (Alpha Chloralose, Rhône Poulenc Santé, France; in doses of 5 mg/kg up to a total of 55 mg/kg). Full anesthesia was maintained with 25–50 mg/kg α-chloralose and 0.1–0.2 mg/kg per hour pentobarbital. Throughout the experiments, respiratory movements were observed and adjustments were made to maintain an adequate amplitude and frequency of inspiration. In order to ensure a constant level of anesthesia, the concentration of α-chloralose was readjusted at the end of each experiment. The cats were paralyzed with tubocurarine (0.1 mg/kg) and artificially ventilated at the rate of 16 breaths per minute, with a tidal volume of 10 ml/kg and an oxygen fraction of 40%.

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Cerebellar stimuli → MLF stimulation → MLF lesion → Cessation

FIG. 1. Hypothetical and presently evidenced pathways between fastigial neurons and motoneurons via contralaterally descending reticulospinal, vestibulospinal or propriospinal tract fibers and commissural interneurons. A: hypothetical pathways and the arrangements of stimulating and recording electrodes. B: neuronal coupling indicated by the results of the present study. FN, fastigial neurons; RS, reticulospinal neurons with axons descending in the medial longitudinal fascicle (MLF); VS, lateral vestibular nucleus neurons; PS, cervical propriospinal neurons; Comm., commissural interneurons; co Mn, motoneurons contralateral to the commissural neurons; Th voll., L voll., descending volleys at the level of the lower thoracic and upper lumbar segments. Arrowheads indicate the sites of stimulation and recording and of the lesions of MLF. Dotted lines indicate the midline.

Electrodes were inserted through cerebellum at an angle of 35° (tip directed rostrally). The initial target positions were the hook bundle of Russell at Horsley–Clarke coordinates: P 9 to 10, L 0.7, H –5; and the LVN at coordinates P 7.5 to 8, L 4–4.5, H –2 to –3, but the final position of all of these electrodes was adjusted on the basis of records of descending volleys from the surface of the lateral funiculus at a Th1–Th13 level, as illustrated in the results. The electrodes were left at sites from which distinct descending volleys were evoked at thresholds of 10–20 μA and were near maximal at 100–150 μA. These stimulation sites were marked at the end of the experiments with electrolytic lesions (0.3 mA for 15 s) and were verified on 100-μm-thick frontal sections of the brain stem. The sections were cut in the plane of insertion of the electrodes using a freezing microtome and were counterstained with cresyl violet. The distribution of the stimulation sites is indicated in Fig. 2A and B (in the cerebellum), E (in the LVN), and F (in the MLF). In three animals the MLF was transected by inserting a narrow spatula (width 3 mm) or a piece of plastic film (0.1 mm thick, 3 mm wide, 10 mm long) into the midline of the medulla a few millimeters caudal to the MLF stimulation site. The plastic film was left in place during the experiment and was removed only after the brain had been fixed by perfusion.

**Stimulation and recording**

Axons of the reticulospinal, vestibulospinal, and fastigial neurons were stimulated using a 0.5-mm electrolytically etched tungsten wire electrode, insulated except for its tip as a cathode and a wire inserted into a neck muscle as an anode. Constant current single, double, or triple stimuli 3.3 or 5.0 ms apart (0.2 ms, 50–150 μA) were used. Peripheral volleys were stimulated with constant voltage stimuli [0.1 ms, intensity expressed in multiples of threshold (T) for the most sensitive fibers in a given nerve].

Intracellular records from motoneurons were made using glass micropipettes (1.5–2.0 μm tip diam) filled with a 2 M potassium citrate solution. The reported results are based on records from 65 motoneurons located in the 4th, 5th, or 7th lumbar segments. Simultaneous records of incoming afferent and descending volleys were taken from the surface of the spinal cord with a silver ball electrode in contact with the dorsal columns close to the dorsal roots entry zone or in contact with the lateral funiculus on the side of location of the motoneurons, usually within 5–10 mm of the microelectrode recording site. DC recording or low-pass filters of 1 Hz were used when recording from motoneurons and both the original records and averages of 10–50 single sweep records were stored (using software designed by E. Eide, N. Pihlgren, and T. Holmström, Dept. of Physiology, Göteborg University). The measurements of amplitudes, areas, and latencies of postsynaptic potentials (PSPs) and descending volleys were made from the averaged records. Student’s t-test was used for the statistical analysis. Records were reproduced using CorelDraw 8.

**RESULTS**

**Relationships between descending volleys following stimulation in the cerebellum, LVN, and MLF**

Stimuli applied at several locations in the midline region of the cerebellum evoked distinct descending volleys at a thoracic level, but those from locations corresponding to the region where a thick bundle of fibers running transversely crossed the midline (the hook bundle of Russell) were largest. Figure 2, A...
Fig. 2. Stimulation sites in the cerebellum, lateral vestibular nucleus (LVN), and the MLF. A: stimulation sites along an electrode track (indicated by the white dashed line) crossing the cerebellum. The electrode crossed the center of the hook bundle of Russell at Horsley–Clarke plane P7, just to the left of the midline, which is indicated by a dotted line. The arrow indicates the site selected for testing interactions between the cerebellar and other stimuli. The scale is in millimeters above and below our reference level. B: cerebellar stimulation sites in 6 other experiments (all at locations corresponding to Horsley–Clarke’s planes P6.5–7.0). C: descending volleys recorded from the surface of the right lateral funiculus at the Th 12 level following stimuli applied at different depths along the electrode track shown in A. The depths with respect to our reference levels and the corresponding Horsley–Clarke’s horizontal coordinates (in parentheses) are shown to the left. D: descending volleys recorded as in C but when stimuli were applied at different Horsley–Clarke rostrocaudal levels at depth H0 (from another experiment). E: stimulation sites along an electrode track (indicated by the dotted line) crossing the LVN at P8. The arrow indicates the site selected for testing effects of LVN stimuli in this experiment. The asterisk indicates LVN stimulation site selected in another experiment (see Fig. 7B). F: MLF stimulation sites in 7 experiments; all were at locations corresponding to Horsley–Clarke’s planes P8.5–9. G: descending volleys recorded as in C, but following stimuli along the electrode track shown in E, and from the site indicated by the asterisk. H and I, field potentials evoked in the LVN at the site indicated by arrow in E; antidromic field potential following stimulation of vestibulospinal tract fibers in the right lateral funiculus at the Th 12 level and monosynaptic field potential following stimuli applied in the cerebellum (shock artifact truncated). J, descending volley recorded as in C but following stimuli applied in the MLF (from the same experiment as records in G). Dotted lines in C, D, and H–J indicate the stimulus artifact and the onsets of the earliest volleys and of the field potentials. Dotted lines in G indicate the stimulus artifact and the onsets of 2 components of volleys evoked from LVN, separated by about 0.5 ms. The early components are attributable to direct stimulation of axons of LVN neurons and the later components to trans-synaptic activation of these neurons (see discussion). Time calibration in J is for all records. Stimulation sites are superimposed on scanned brain stem sections (A, E, and F) and on the photomicrograph of the region of the hook bundle (B). FN, fastigial nucleus; H-C, Horsley–Clarke; Pyr, pyramidal tract; P, posterior; RB, restiform body; SO, superior olive; TB, trapezoid body; 7G, genu of the facial nerve.

and C, illustrates this with data from an experiment in which the most distinct volleys were evoked from depths labeled +0.5 to −1 (corresponding to Horsley–Clarke’s coordinates H +1.2 to 0), the site selected by an arrow in Fig. 2A having been selected for testing effects of the cerebellar stimulation. Stimulation sites selected in other experiments are indicated in Fig. 2B. When the electrode was inserted at different rostrocaudal levels, the largest volleys were evoked from locations corresponding to Horsley–Clarke coordinates P 6.5 to 7 (Fig. 2D) and again were restricted to the region of the hook bundle of Russell.

Latencies of descending volleys evoked by cerebellar stimuli (2.66 ± 0.08 ms; mean ± SE; n = 7) were about 0.8 ms longer than latencies of descending volleys evoked by MLF stimuli (1.86 ± 0.04 ms; n = 7) and about 0.7 or 0.2 ms longer than latencies of descending volleys evoked by LVN, as illustrated in Fig. 2, C and G. Because fastigial neurons have been found to activate reticulospinal as well as vestibulospinal neurons (Alstermark et al. 1987a,b; Homma et al. 1995; Mori et al. 1998), effects of cerebellar stimuli could be relayed by either or both of these descending tract fibers. The following attempts were therefore made to estimate to what extent the reticulospinal and vestibulospinal tract neurons might contribute to the descending volleys evoked from the cerebellum.

First a comparison was made of the spinal rostrocaudal distribution of the descending volleys of the cerebellar, LVN,
and MLF origin. Figure 3.A–C, shows that the cerebellar and LVN volleys were distinct rostral to the L4 segment but became difficult to detect at more caudal levels (as previously found for LVN volleys by (Krutki et al. 2003), whereas early components of the MLF volleys remained as distinct at lower as at upper lumbar levels and their second component (marked by an asterisk) increased, in particular at the L5–L7 levels (Jankowska et al. 2003). Similar differences were found in all three experiments in which they were analyzed. The parallel distribution of the cerebellar and LVN volleys and more caudal extent of the late component of the MLF volleys are thus more in keeping with the cerebellar volleys reflecting activity in the vestibulospinal than in the reticulospinal tract fibers.

Second, we verified that cerebellar and LVN stimuli continued to evoke descending volleys after a lesion in the medial part of the brain stem a few millimeters caudal to the MLF stimulation site. As illustrated in Fig. 3F, the lesion abolished descending volleys from MLF but did not interfere with volleys evoked by the cerebellar and LVN stimuli.

Third, the following tests were made to examine the possibility that effects of cerebellar stimuli were relayed by the same fibers that were stimulated within the LVN but not by fibers stimulated in MLF. If the cerebellar stimuli did activate the same fibers, then these fibers could not be activated twice when intervals between the stimuli were very short. The minimal effective intervals should be longer than the refractory period following activation of vestibulospinal or reticulospinal tract neurons. In the case of LVN stimulation, they should in addition be longer than twice the conduction time between LVN and the cerebellum during which nerve impulses that are orthodromically and antidromically conducted along axons of fastigial neurons would collide. Stimuli to the LVN or MLF were therefore applied within 2 ms before or after the cerebellar stimuli. The stimuli were near maximal (100–200 μA) and they were expected to activate fibers within a radius of 1–2 mm (Gustafsson and Jankowska 1976) but without spread of current to the other stimulation sites. Records in Figs. 2, C, G, and J and 3D argue against such a spread of current since minimal latencies of the earliest components of the cerebellar, MLF, and directly induced LVN volleys always differed, when stronger as well as when weaker stimuli were used.

In Fig. 4 the compound volleys evoked by joint actions of pairs of the cerebellar, LVN, and MLF stimuli (labeled “both”) are compared with the sums of the volleys evoked by each of

FIG. 3. Comparison of descending volleys of cerebellar, LVN, and MLF origin. A–C: descending volleys recorded from the right lateral funiculus at the Th12 level and from the left lateral funiculus at the three indicated spinal levels (all at the same amplification). The asterisk in C indicates the second component of the MLF volleys, which is larger in more caudal segments (Jankowska et al. 2003). D and F: comparisons of volleys evoked by stimuli at 2 different intensities applied within MLF, LVN, and cerebellum in another experiment before (D) and after (F) a lesion of MLF (E) made a few millimeters caudal of the MLF stimulation site. The gray trace superimposed on the second trace in F shows the prelesion MLF volley. Records in D were obtained at the beginning of the experiment when stimulus shock artifacts were larger than at the end. Vertical dotted lines indicate points of transition between the positive and negative phases of the first components of the 3 volleys evoked by 100 μA. Note that albeit 300 μA evoked LVN volleys at shorter latencies, these were longer than of MLF volleys (i.e., they were not compatible with current spread to MLF). In contrast, shorter latencies of volleys evoked by 300 μA cerebellar stimuli are compatible with current spread to the LVN. CU, cuneate nucl; IO, inferior olive; 5S, spinal trigeminal nucl.
them separately. When the same fibers were stimulated twice at a very short interval, as in Fig. 4F, the differences (bottom traces) were as large as the volleys evoked by either the first or the second stimulus, indicating that the fibers activated by the first stimulus were refractory to the second stimulus. The large difference in Fig. 4A indicates thus that the descending volleys evoked by the cerebellar stimuli may have been relayed by LVN neurons that were made refractory to the LVN stimuli. Descending volleys of cerebellar origin may accordingly reflect activity in axons of vestibulospinal neurons that were synaptically activated by fastigial neurons. In contrast, there was hardly any difference when the LVN stimuli were delivered at a longer interval (Fig. 4B), indicating that at this interval the LVN neurons could be activated by both stimuli. The same effects were seen in experiments in which LVN volleys were induced indirectly or were induced both directly and indirectly. In similar tests with other combinations of stimuli, the volleys summed linearly: between the cerebellar and LVN stimuli (Fig. 4A; in 4 experiments) or between the LVN and MLF stimuli (Fig. 4E; in 2 experiments). This indicates that the descending volleys evoked by these stimuli reflected activity in distinct fibers activated by them.

Taken together these observations indicate that any early effects of fastigial neurons on lumbar contralateral motoneurons are relayed by vestibulospinal rather than by reticulospinal neurons with axons in MLF, as indicated in Fig. 1B.

Effects of cerebellar stimuli on motoneurons

Effects of cerebellar stimuli were analyzed in a sample of 65 motoneurons recorded in the L4th, 5th, and 7th segments. The motoneurons included 12 PBST, 35 GS, 11 Q, and 7 Sart motoneurons, all with action potentials 50–75 mV and with stable membrane potential during the whole period of recording. When trains of three stimuli near maximal for the cerebellar descending volleys were applied, EPSPs or IPSPs following these stimuli were found in only a proportion of the motoneurons. EPSPs were evoked in 26 (PBST, GS, and Q) and IPSPs in 9 (Sart and GS) motoneurons, totally in 54% of 65 motoneurons tested. EPSPs or IPSPs from LVN were evoked in 6 and 4, totally 30% of 34 motoneurons tested. In contrast, EPSPs or IPSPs from MLF were evoked in all 40 motoneurons tested. As illustrated in Fig. 5, both the EPSPs and IPSPs of cerebellar origin were in addition much smaller than those evoked from MLF in the same motoneurons. The minimal PSPs that repeatedly followed cerebellar stimuli at the same latency were of about 0.1 mV in averaged records and 0.1–0.2 mV in single sweep records. Their maximal amplitudes were within the same ranges (0.1–0.5 mV) as those of EPSPs and IPSPs of LVN origin (0.1–0.3 mV). Greater numbers of cerebellar and LVN stimuli were always needed to evoke EPSPs or IPSPs compared with MLF stimuli. The separation between dotted lines in Fig. 5A shows in addition that the latency of EPSPs evoked by cerebellar stimuli (5.31 ± 0.04 ms; mean ± SE) was about 1 ms longer than the latency of EPSPs from MLF (4.18 ± 0.08 ms; P < 0.001) and similar to the latency of EPSPs from LVN (5.36 ± 0.07 ms; P > 0.5) when measured from stimulus artifacts. Differences in latencies of the IPSPs were also of about 1 ms (Fig. 5, D and E). These data are thus compatible with two relay neurons between the fastigial neurons and contralateral motoneurons compared with only one relay neuron between MLF fibers and these motoneurons (Jankowska et al. 2003; Krutki et al. 2003).

The small proportion of motoneurons in which PSPs were evoked by cerebellar stimuli and the small size of these PSPs might reflect a weak connection between fastigial neurons and
hindlimb motoneurons. However, the results presented in the following sections will show that, when cerebellar stimuli were appropriately timed with LVN stimuli, they induced EPSPs or IPSPs in a very high proportion of motoneurons, indicating that the majority of contralateral motoneurons are potentially affected by fastigial neurons. Weak effects of cerebellar stimuli applied alone may therefore depend on a relatively low level of excitability of the involved relay neurons under our experimental conditions.

Interactions between the synaptic actions of cerebellar and LVN stimuli on motoneurons

Figure 6B illustrates effects of combining cerebellar and LVN stimuli, both of which failed to evoke any EPSPs when applied alone (Fig. 6, A and C) on a motoneuron. When the stimuli were separated by 1 ms or more, they evoked a distinct EPSP but the mutual facilitation between their actions disappeared when one stimulus fell into the collision/refractory period following the other one. The time course of this facilitation is plotted in Fig. 6G, a similar plot for another motoneuron shown in Fig. 6H. A similar time course of facilitation was found in all 19 motoneurons in which it was investigated.

The dominant effect was most often an increase in the amplitude of EPSPs evoked by the cerebellar stimuli, as illustrated in Fig. 6B, as if the stimuli applied within the LVN decreased the threshold for synaptic activation of LVN neurons by fastigial neurons. This is indicated by the constant latency of the EPSPs with respect to the cerebellar stimuli and their independence of the timing of the LVN stimuli. In addition the latencies of the EPSPs evoked at the first three intervals (1.2–3.1 ms) in Fig. 6B were too short for the EPSPs to be evoked by LVN volleys. Increases in the amplitude of EPSPs of LVN origin were also seen, indicating a decrease in threshold for activation of LVN neurons by cerebellar stimuli following fastigial stimuli.

Similar facilitation, and with a similar time course, occurred in six motoneurons when the cerebellar and LVN stimuli evoked IPSPs (Fig. 6, D–F and I). Facilitation of both EPSPs and IPSPs was maximal when cerebellar stimuli were applied in the center of the area of the hook bundle of Russell and when LVN stimuli were applied within the limits of the LVN (Fig. 7).

Transection of reticulospinal tract fibers in the MLF did not prevent a mutual facilitation between effects of stimuli applied in the cerebellum and in LVN. The facilitation was found in all six motoneurons tested after an MLF lesion and its time course was similar to that in motoneurons illustrated in Fig. 6, G–I.

Interactions between cerebellar and MLF synaptic actions on motoneurons

As shown above, the operation of reticulospinal pathways is not a prerequisite for fastigial actions on contralateral motoneurons and it appears unlikely that trisynaptic actions of fastigial neurons would be in a significant way relayed by reticulospinal neurons with axons in MLF. Reticulospinal tract neurons that are activated by fastigial neurons via other neurons might nevertheless increase the probability of activation of commissural interneurons mediating actions of fastigial and LVN neurons. To investigate this possibility we analyzed mutual facilitation between the cerebellar and MLF actions on motoneurons in the same way as facilitation between the cerebellar and LVN actions.

Figure 8 shows that the facilitation following cerebellar and MLF stimulation was as potent as that following cerebellar and LVN stimulation and that it involved both EPSPs and IPSPs: it was found in all 25 and 15 motoneurons tested, respectively. However, an important difference was found in the time course of this facilitation. As shown in Fig. 8, G and H, it was always most potent when the MLF and cerebellar volleys coincided or followed each other within a fraction of a millisecond (intervals indicated by thick lines above the abscissa). This time course was found for facilitation of all 14 EPSPs and all 4 IPSPs analyzed, both when single (Fig. 8, B and C) and double (Fig. 8, E and F) MLF stimuli were used. The second (spinally relayed) components of the descending volleys, indicated by arrowheads in Fig. 8B, were particularly large at these intervals. They thus strongly relate the facilitation with more potent activation of commissural interneurons (for arguments that these components represent a spinally relayed event, see Jankowska et al. 2003). The strongest facilitation of both EPSPs and IPSPs of MLF origin was evoked from the center of the area of the hook bundle of Russell, as in the experiment illustrated in Fig. 7, A and D.

Discussion

This study reveals that neurons in the fastigial nuclei may coordinate movements of the left and right extremities minimally via only two relay neurons, the first relay neurons in the brain stem and the second relay neurons in the spinal cord. To simplify the analysis, the fastigial actions were investigated in preparations in which only one-half of the spinal cord was left intact, which allowed the supraspinal relay neurons involved to be restricted to those with axons descending on only one side of the spinal cord.
The effects evoked by cerebellar stimulation can be attributed to the axons of fastigial neurons since these effects were evoked from within the width of the midline cerebellar white matter through which these axons cross and the effects of stimuli of 50–100 μA (with the estimated spread of current of 0.5–1 mm) declined when the stimulating electrode was moved more than about 0.5 mm above or below the hook bundle of Russell. In addition, stimuli delivered more than 1 mm more rostral or caudal to this site evoked much weaker effects. The most effective stimulation sites overlapped with the areas from which retrograde labeling of fastigial neurons (Homma et al. 1995) and anterograde labeling of fastigiofugal fibers projecting to the nucleus reticularis gigantocellularis and magnocellularis and to vestibular nuclei (Homma et al. 1995; Mori et al. 1989, 1998, 1999; Nakajima et al. 2000).

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The effects of stimuli applied in MLF and LVN are attributed to axons of reticulospinal and vestibulospinal tract neurons relying on previous control experiments (Jankowska et al. 2003; Krutki et al. 2003). In addition, to minimize the spread of current, we used the weakest effective currents and always verified that effects induced by stimuli applied within MLF or LVN were not reproduced when the stimulating electrodes were moved outside these structures. Transsynaptic activation of other neurons via axon collaterals of either reticulospinal or vestibulospinal neurons, or of neurons providing input to them, could not be excluded. However, the probability of actions evoked by axon collaterals of reticulospinal neurons (e.g., Mitani et al. 1988a–c) or of neurons stimulated within LVN was reduced by the demonstration that the main reported
effects of reticulospinal neurons disappeared after a lesion of MLF within the more caudal part of the medulla, whereas those from the cerebellum and LVN remained. For discussion of other methodological problems related to stimulation of MLF and LVN see Jankowska et al. (2003) and Krutki et al. (2003).

Reticulospinal versus vestibulospinal relay of fastigial actions

Reticulospinal neurons were expected to be involved in mediating actions of fastigial neurons in view of the previous evidence for a strong coupling between neurons in the fastigial nuclei and at least some neurons in the reticular formation (Mori et al. 1998). However, several of our observations argue against the mediation of the most direct fastigial actions by reticulospinal neurons with axons in the MLF. The most decisive of these observations was that, when the descending volleys following MLF stimuli were abolished by a lesion of the MLF at a more caudal level, LVN and cerebellar stimuli continued to evoke descending volleys (Fig. 3) as well as trisynaptic EPSPs and IPSPs in motoneurons. The lack of collision or of any interactions due to refractoriness of the MLF fibers when the cerebellar and MLF stimuli were applied in quick succession (Figs. 4 and 8) are likewise incompatible with the activation of MLF fibers by stimuli applied in the hook bundle of Russell.

In contrast, the interactions between effects of the cerebellar and LVN stimuli applied during collision and/or refractory periods following one of them (Figs. 4 and 6) were what would be expected for effects mediated via the same fibers. The similar appearance of the descending volleys and cord dorsum potentials evoked by the cerebellar and LVN stimuli recorded in the midlumbar segments is also in keeping with this possibility and, as shown in Fig. 3, clearly differs from the appearance of the volleys evoked from MLF. The question of the relative contribution of vestibulospinal and propriospinal neurons (see Fig. 1) to the descending volleys of cerebellar origin after MLF lesions, and to fastigial actions on contralateral motoneurons, has not been addressed in this study and remains open.

Spinal relay of fastigial actions on hindlimb motoneurons

Previous studies have shown that vestibulospinal and reticulospinal neurons excite or inhibit contralateral motoneurons both via single interposed spinal commissural interneurons, i.e., dysynaptically and polysynaptically (Jankowska et al. 2003; Krutki et al. 2003). Since the earliest components of the EPSPs and IPSPs evoked by cerebellar stimuli were induced at latencies that were only about 1 ms longer than of the earliest components of PSPs of MLF origin, they might have been evoked trisynaptically. EPSPs and IPSPs mediated via vestibulospinal tract fibers would be relayed by commissural interneurons located on the same side of the spinal cord in view of ipsilateral terminal branching areas of these fibers in the lumbosacral enlargement (Holstege and Kuypers 1982; Kuze et al. 1999). Reticulospinal neurons that are either directly or indirectly activated by fastigial neurons could act via the same interneurons (Krutki et al. 2003; Skinner and Remmel 1978) but they could also activate interneurons on the opposite site of the spinal cord, via crossed local axon collaterals of reticulospinal tract fibers (Matsuyama et al. 1988, 1997, 1999; Peterson et al. 1975). However, since no evidence for the contribution of reticulospinal neurons with axons in MLF to fastigial
actions investigated has been found in this study, our conclusions can be restricted to fastigial actions mediated by commissural interneurons. Commissural interneurons that are monosynaptically excited from MLF and LVN and project to contralateral motor nuclei (Krutki et al. 2003) form synaptic contacts with motoneurons and their terminals contain either glutamate or glycine (Bannatyne et al. 2003b). Subpopulations of these neurons might thus mediate disynaptic excitation or inhibition of reticular and vestibulospinal origin and be interposed in trisynaptic excitatory and inhibitory pathways between fastigial neurons and contralateral motoneurons as indicated in Fig. 1B.

Functional consequences

We wish to stress two main corollaries of the network of neurons mediating actions of fastigial neurons on contralateral motoneurons outlined above. The first is that fastigial neurons may coordinate movements of the two hindlimbs with movements of the forelimbs, trunk, and head via a minimal number of additional spinal relay neurons. The second is that adjustments of postural reactions by fastigial neurons via vestibulospinal and reticulospinal descending systems involve integration of descending commands that to a great extent occurs at a spinal level. Our results show that commissural interneurons with direct actions on motoneurons are important in this integration. However, when the spinal cord is intact, and not hemisected as in preparations used in this study, other neurons may be involved. These neurons might, e.g., include interneurons with input from vestibulo- and reticulospinal neurons, located at the same side as the motoneurons or providing polysynaptic input to commissural interneurons. These possibilities have not been included in the diagram of Fig. 1 but might be considered in future studies.

A discussion of consequences of the apparently negligible contribution of reticulospinal neurons with axons in MLF to the most direct actions of fastigial neurons in the lumbar segments and their role in mediating locomotion induced by cerebellar stimuli (Mori et al. 1998, 1999) is outside the scope of the present study. However, our results indicate that it would be interesting to compare the role of different populations of
reticular and vestibular neurons and of their target neurons in the effects of such stimuli.

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REFERENCES


