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Locomotor Stepping Elicited by Electrical Stimulation of the Lateral Hypothalamus Requires an Ipsilateral Descending Pathway[1]

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SINNAMON, H. M., S. H. LEE, D. B. ADAMS AND C. K. STOPFORD. *Locomotor stepping elicited by electrical stimulation of the lateral hypothalamus requires an ipsilateral descending pathway.* PHYSIOL BEHAV 33(2) 209–215, 1984.—The effects of unilateral midbrain lesions on stepping produced by ipsilateral medial forebrain bundle (mfb) stimulation was determined in acute experiments. Rats were anesthetized with nembutal, mounted in a stereotaxic apparatus and suspended over a wheel which rotated when the rat stepped. Stimulation consisted of 10-sec trains of 50 Hz cathodal pulses (0.5 msec) at currents up to 200 μA delivered through monopolar electrodes. The stimulation sites were in the mfb at the level of the subthalamic nucleus. One side of the midbrain was randomly selected to receive a radio-frequency lesion. Certain midbrain lesions (N=11) abolished or severely reduced stepping elicited from ipsilateral mfb stimulation but produced no effect on contralaterally elicited stepping. Areas of common damage in these lesions included the medial half of the medial lemniscus and the ventral tegmental area (VTA). Lesions that were without effect (N=18) generally spared the dorsal VTA but damaged extensively either the ventrolateral VTA, medial lemniscus, substantia nigra, red nucleus, or central gray. A supplemental experiment further implicated a critical descending system since neither unilateral nor bilateral mfb lesions blocked stepping elicited by VTA stimulation. Together these results suggest that a descending path through the ipsilateral dorsal VTA mediates stepping elicited by mfb stimulation in the anesthetized rat.

Locomotion Brain stimulation Medial forebrain bundle Lateral hypothalamus Ventral tegmental area Lesions

WELL coordinated locomotor stepping movements can be elicited by electrical stimulation of the diencephalon, midbrain, and lower brainstem of the anesthetized rat [8, 9, 14, 15]. The hypothalamic areas that are effective include the dorsomedial and posterior hypothalamic nuclei in the medial hypothalamus and the medial forebrain bundle (mfb) in the lateral hypothalamus. In the case of the mfb at least, there is reason to hypothesize that descending projections are responsible for the stimulation-elicited stepping. Projections from the lateral hypothalamus and the mfb descend to certain midbrain areas [4, 11, 17] where electrical stimulation also elicits stepping [15]. Descending projections are also implicated by the finding that in cats with transections through the rostral thalamus, stimulation of the posterolateral hypothalamus produces stepping [12]. Locomotor approach is an important component of predatory attack by the cat. Lesions in lateral hypothalamic sites where stimulation produces predatory attack result in anterograde degeneration that corresponds with the descending mfb projections to the midbrain [1]. Locomotor approach is also an important correlate of the rewarding effects of stimulation in the lateral hypothalamus [2]. Although the locomotor component has not been studied, it appears that at least the rewarding effect produced by hypothalamic stimulation survives removal of the telencephalon [5].

This study was intended to test the notion that stepping elicited in the anesthetized rat by stimulation of the mfb is mediated by descending pathways. A further goal, given evidence for a descending system, was to localize the midbrain region in which the system coursed. The general plan was to elicit stepping by mfb stimulation, make a lesion of the ventromedial midbrain ipsilateral to the stimulation site, and determine if an impairment resulted. Since the descending mfb projections are primarily uncrossed [4,11,17], we used a stimulation site in the contralateral mfb which also supported stepping as a control for nonspecific factors that might reduce stepping such as postural changes, tonus, and anesthetic effects. Evidence inconsistent with the involvement of a necessary ascending system was obtained from an experiment in which lesions in the mfb were found not to affect stepping elicited by stimulation of the ventromedial midbrain.

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METHOD

Subjects

Male Sprague-Dawley rats (N=28) weighing between 237 and 470 grams were used. They were housed in group cages on a reversed light cycle; experiments were performed during their dark cycle.

Surgery

The rats were anesthetized by means of intraperitoneal injections of nembutal (50 mg/kg) and mounted in a Kopf stereotaxic apparatus such that Bregma and Lambda were on the same horizontal plane. Local injections of Xylocaine were made around the incision. Four holes of 1-mm diameter were drilled over the lateral hypothalamus and ventral tegmental area through which were passed the stimulation or lesion electrodes. Four additional holes were drilled to receive stainless steel screws which served as leads for the anodal return line to the stimulator and the reference line for the lesion-maker. The hypothalamic coordinates were 3.0–3.5 mm posterior to Bregma, 1.3 mm lateral to midline and 7.5–8.3 mm ventral to the dural surface. Midbrain coordinates were 5.0–5.5 mm posterior, 1.0 mm lateral and 8.4 mm in the deepest case. Supplemental anesthesia was provided by intraperitoneal injections of nembutal at a dose of 7.5 mg/kg as needed (usually hourly). Injections of the supplemental anesthesia were given when the rat displayed a characteristic pattern of behaviors marked by active sniffing, bilateral forelimb extension, flexion of the trunk and pelvis, and hindlimb extension. Body temperature was monitored with a rectal thermometer and maintained at 37–38°C with a heat lamp.

Apparatus

The rat's head was secured by ear bars and a nose clamp, the body was supported by a sling under the thorax and abdomen and the base of the tail was centered between the two side rails of the stereotaxic apparatus by means of masking tape. The forelimbs and hindlimbs hung freely and the paws contacted the outer surface of the circumference (width of 10 cm) of a 30-cm diameter wheel. Stepping movements principally by the hindlimbs caused the wheel to revolve. Revolutions were detected by a magnetic switch (resolution, 1/6 of a revolution) and were recorded on electromagnetic counters during the train of stimulation. The relationship of wheel counts to stepping varied between rats as a function of size and within rats as a function of extent of flexion movement, force of the extension movement and frequency of the step cycle. However, in the most typical cases, one wheel count was approximately equivalent to one step cycle of the hindlimbs.

Stimulation was provided by a Haer Pulsar IV constant current stimulator. Ten-sec trains of 50 Hz, 0.5 msec cathodal pulses were delivered on each trial. The stimulation and lesion electrodes were fashioned from No. 00 stainless steel insect pins insulated with Epoxylite. Stimulation electrodes were cut to expose the cross-section of the tips. Lesion electrodes had 0.5 mm of the tip exposed. Lesions were made with a Grass radio-frequency generator set for 40–60 sec at 2–8 mA (depending upon the size of lesion desired).

Procedure

There were two types of experiments, those with caudal lesions and those with rostral lesions, and each type had a prelesion and postlesion phase. In the experiments with caudal lesions, stimulation electrodes were placed in the lateral hypothalamus and lesions were made in the midbrain. In the experiments with rostral lesions, the locations were reversed.

In the prelesion phase of either type of experiment, stimulation electrodes were implanted at bilateral sites, each of which supported electrically-elicited bilateral hindlimb stepping at currents of 100 μA or less. Typically one or both forelimbs stepped also. The stimulation electrodes were fixed to the skull with dental acrylic. Next a lesion electrode was implanted unilaterally on a randomly selected side. Baseline measures of stimulation-elicited stepping were taken and the lesion was made.

In the postlesion phase, each stimulation site was tested at approximately 10-min intervals. The test at a given current level began with a warm-up trial which was followed within 5 sec by two trials in which wheel revolutions during the train were counted. The limbs involved in stepping and any other elicited behavior were noted. The details of the testing procedure depended on the effects of the lesion. Generally the current level used in baseline determinations was also used in the postlesion phase but if any decrease in stepping was noted, a range of currents between 25–200 μA was tested. If revolutions elicited by ipsilateral stimulation at the current used for baseline determination exceeded 50% of prelesion baseline on any trial, the lesion was considered ineffective and a second lesion was usually made either at the same site at a higher current level (up to 8 mA) or at a new site. If the revolutions produced by ipsilateral stimulation were consistently reduced below 50% of the prelesion baseline, the lesion was considered effective and testing continued for approximately 1 hour to allow sampling at various levels of anesthesia.

The criterion of at least a 50% reduction on all postlesion trials was stringent but necessary because of the variability of the stepping measures. Stimulation-elicited stepping is strongly influenced by the depth of anesthesia but in no simple way. In some cases stepping became progressively stronger as the level of anesthesia declined, but in other cases the increased extensor tonus associated with decreased anesthesia seemed to interfere with coordinated stepping. Consequently, maximal stepping appeared midway between the hourly supplemental injections. Therefore to demonstrate that a reduction in stepping was actually due to a lesion it was necessary to allow for the full range of variability expected in the complete cycle of supplemental nembutal.

Histology

When testing was complete the rat was injected with a lethal dose of Chloropent and perfused through the heart with normal saline followed by Formalin. The brain with electrodes in place was fixed in Formalin for several days and 64 micrometer transverse sections were taken on a cryostat microtome. The sections were stained with cresyl violet and examined with a Bausch and Lomb projector at 23 magnifications. The electrode and lesion locations were projected onto drawings adapted from the atlas of Paxinos and Watson [13].

RESULTS

Caudal Lesions

These data were drawn from 24 rats in which stepping

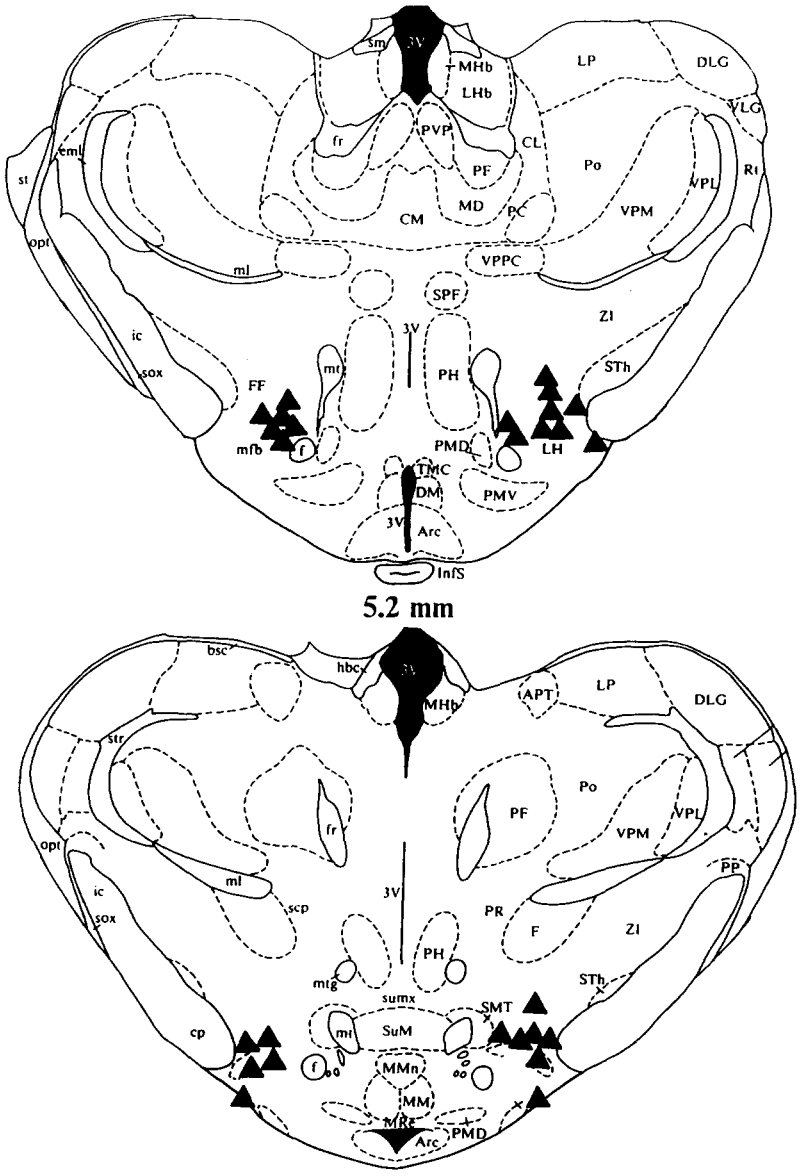

FIG. 1. Schematic illustration of the stimulation sites in the medial forebrain bundle (mfb) at which stimulation produced locomotor stepping. Sites represented in the left mfb were ipsilateral to the completely or partially effective lesions shown in Fig. 2. Those represented on the right were ipsilateral to the ineffective lesions shown in Fig. 3. All drawings were adapted from the atlas of Paxinos and Watson [13]; the distance anterior to earbar zero is given below each drawing.

was elicited from stimulation electrodes located within the medial forebrain bundle (mfb) ipsilateral to a lesion of the midbrain. The stimulation sites are represented by black triangles in Fig. 1. On the left side of the figure are illustrated those sites at which stimulation-elicited stepping was reduced by the ipsilateral lesion. On the right are shown the sites at which stimulation-elicited stepping was unaffected by the ipsilateral lesion. There was somewhat more scatter among the stimulation sites of the unaffected group which appeared to be attributable to its larger size (16 sites vs. 11 sites). Otherwise there seemed to be no differences between the regional patterns of the two groups. Stimulation sites that were contralateral to the lesion which served as controls are not shown.

Certain lesions (N=11) were effective in reducing stepping produced by stimulation of the ipsilateral mfb without affecting contralaterally elicited stepping. Figure 2 illustrates these lesions as they appeared in their maximal extent. Five lesions produced a virtually complete block of effective stepping, i.e., no wheel counts were detected. In most of these cases, observable stepping movements were completely absent at the standard and lower current levels; the hindlimbs in these cases showed some movement or tension and the rat typically showed an increased respiration rate. At

currents higher than standard (up to 200 μA), it was occasionally possible to elicit stepping but it was always less than 50% of the prelesion baseline wheel counts. The five lesions are among those illustrated in Fig. 2. They are lesion 4 at AP 2.7 mm, lesions 7,9, and 26 on AP 3.2 mm and lesion 31 on AP 3.7 mm. The mean prelesion wheel counts in this group ranged from 6.5 to 24.5 with a median of 12.

The remainder of the lesions (N=6) illustrated in Fig. 2 severely reduced stepping elicited by ipsilateral mfb stimulation but not to the point of completely preventing wheel counts. Some visually detectable stepping was present and it was sufficient to produce movement of the wheel on at least one postlesion trial. In all of these cases, the counts produced by stimulation at standard or lower currents were less than 50% of prelesion levels on all test trials. Higher currents than the standard level (up to 200 μA) sometimes elicited wheel counts higher than 50% of the prelesion baseline. This group included lesions 22, 28–30 at AP 3.7 mm and lesions 14 and 21 at AP 3.2 mm. The mean prelesion baseline counts for the group ranged from 13 to 32 with a median of 17.

As may be seen in Fig. 2, the effective lesions varied considerably in size but generally did involve certain common structures. Because some of the smallest lesions, e.g. 4, 7 and 31 were most effective, it appeared that location rather than extent was the shared feature of these lesions. All effective lesions damaged the ventral tegmental area (VTA) and the medial aspect of the medial lemniscus (ml). In 10 of 11 cases, the damage occurred at AP 3.7 mm. This is obvious for the five lesions illustrated at AP 3.7 mm but it was also true for the five lesions with their centers at AP 3.2 (middle of Fig. 2). All of the lesions illustrated at AP 3.2 mm extended into the VTA at AP 3.7 mm but only lesion 22 of the five with centers at AP 3.7 mm extended to AP 3.2 mm. This pattern indicates that damage to the VTA and adjacent medial lemniscus at AP 3.7 mm was sufficient to impair stepping elicited by ipsilateral mfb stimulation. However, it should be noted that lesion 4 damaged the VTA principally at AP 2.7 mm; it extended to AP 3.2 mm but not to AP 3.7 mm. This case is consistent with the apparent requirement for damage to the VTA and adjacent medial lemniscus but it suggests that the damage need not include the most anterior levels in all cases.

The lesions without effect on ipsilaterally or contralaterally elicited stepping are illustrated in Fig. 3. The vast majority of these lesions were smaller than the effective lesions and damaged structures that were dorsal to areas implicated in Fig. 2, i.e., the VTA and adjacent medial lemniscus. However, four lesions (No. 18, 19, 23 and 32) damaged that area and nevertheless were without detectable effect. One of these lesions, 32, was considerably smaller than the effective lesions but the others were comparable in size and extent of damage at AP 3.2 mm. However, none of them involved significant damage at AP 3.7 mm. Neither lesion 19 nor 23 involved any detectable damage to the VTA at AP 3.7 mm and the damage associated with lesion 18 was confined only to the most ventral aspects of the VTA, failing to extend as dorsally as the medial lemniscus. The ineffective lesions then, were less extensive in anterior extent than the effective lesions.

In summary, lesions that blocked or significantly reduced stepping produced by ipsilateral stimulation of the mfb all extensively damaged the VTA particularly the area coextensive with the medial aspect of the medial lemniscus. Lesions that did not impair stepping either spared this region

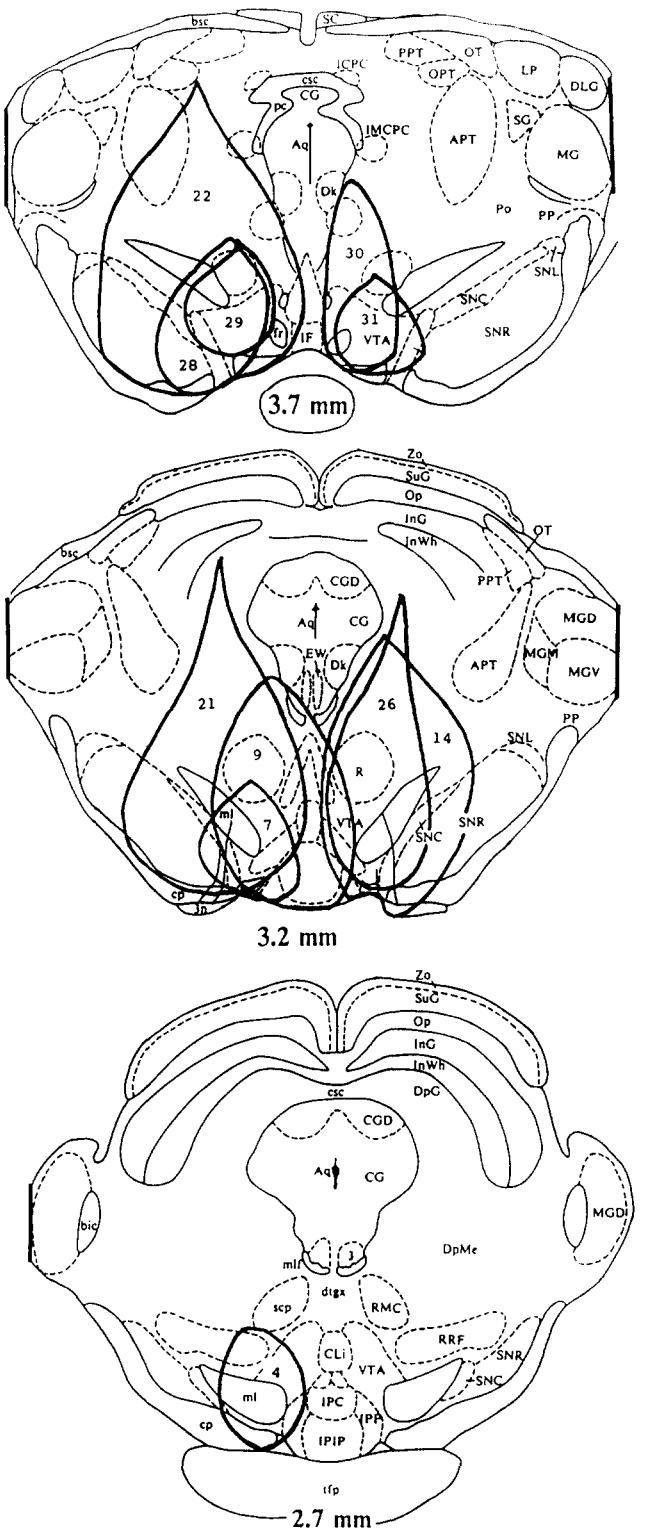

FIG. 2. Schematic representation of 11 lesions which impaired stepping elicited by ipsilateral mfb stimulation but were without effect on the stepping elicited by contralateral mfb stimulation. In this figure and in Fig. 3, the side of the brain on which the lesion is illustrated is arbitrary. Lesions 4, 7, 9, 26 and 31 reduced postlesion wheel counts to zero on all test trials at the standard current levels. The remaining lesions severely reduced stepping such that on no test trial with the standard current level were wheel counts greater than 50% of the prelesion levels.

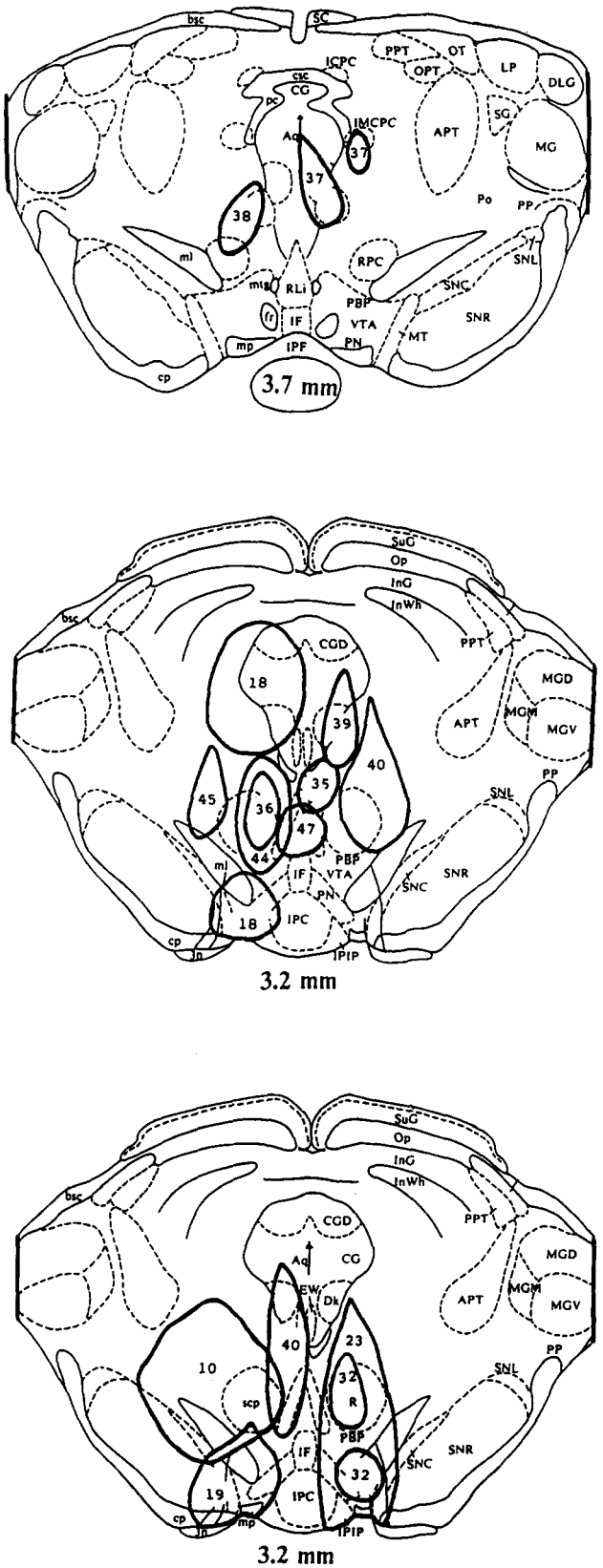

FIG. 3. Illustrations of lesions that were without effect on stepping elicited by ipsilateral stimulation of the mfb. In some cases, two lesions were made in one rat and the same number appears in each representation.

entirely or damaged it to a relatively limited anterior posterior extent.

Rostral Lesions

If stepping elicited by mfb stimulation is mediated by a descending system coursing through the VTA as the lesions illustrated in Figs. 2 and 3 suggest, then stepping elicited by VTA stimulation should also involve this system and it should be insensitive to lesions placed rostrally in the mfb. To test this notion, we used the same approach as in the first experiment but reversed the locations of the stimulation and lesion electrodes.

Figure 4, top, illustrates the locations of stimulation electrodes in the VTA and nearby medial lemniscus that supported stepping. These sites are consistent with the pattern of effective sites found in a previous study [15]. Two rats had two effective electrodes (one on each side) and two rats had only one effective electrode. The mfb lesions corresponding to these stimulation sites are shown in the lower panels of Fig. 4, e.g., lesion 3L was tested with stimulation site 3L. All of the lesions extensively damaged the mfb. Two were less than complete, cases 3L and 3R shown in the middle of Fig. 4, but they did damage the dorsal half of the mfb, including its most lateral parts. None of the lesions reduced stepping produced by VTA stimulation below prelesion levels. One possible exception was case 1L in which near baseline levels of stepping were seen in the first test after the lesion but not thereafter. The stimulation site in this case was at the ventral surface on the midbrain and it is possible that the electrode tip passed out of the brain after the electrode was fixed to the skull. The stimulation sites 1R, 3R and 3L were tested in the presence of bilateral lesions of the mfb which were no more effective than unilateral lesions.

DISCUSSION

Stepping elicited by mfb stimulation in the anesthetized rat requires an intact ventral tegmental area on the same side. The most likely explanation for this finding is that descending fibers from the mfb mediate the response and they pass through or terminate in this area. Moreover, if the mfb fibers do synapse in this area, then the postsynaptic system would appear to project caudally also, since stepping elicited by VTA stimulation was unaffected by rostral lesions in the mfb. Comparison of completely effective, partially effective and ineffective lesions indicates that the systems involved in stepping are localized only to a limited extent in the ventromedial midbrain. There appeared to be a joint requirement for the completely effective lesion to be located in the VTA, particularly in its dorsal part, and to be sufficiently large. This requirement is quite consistent with the projection patterns of mfb fibers and terminals which are dense in the dorsal VTA but also fan out laterally through the ventral to the medial lemniscus [4, 11, 17]. The findings that some lesions had to be very large in order to completely block stepping and that a few relatively large lesions involving the VTA had less than complete effects suggest that multiple components of the mfb with different midbrain trajectories are involved in locomotor initiation. It is conceivable that when the mfb stimulation activates few of these systems, a small midbrain lesion would be more likely to interrupt the relevant fibers. When the mfb stimulation activates several of them, the lesion would have to be proportionately larger.

The finding that VTA lesions block stepping elicited by

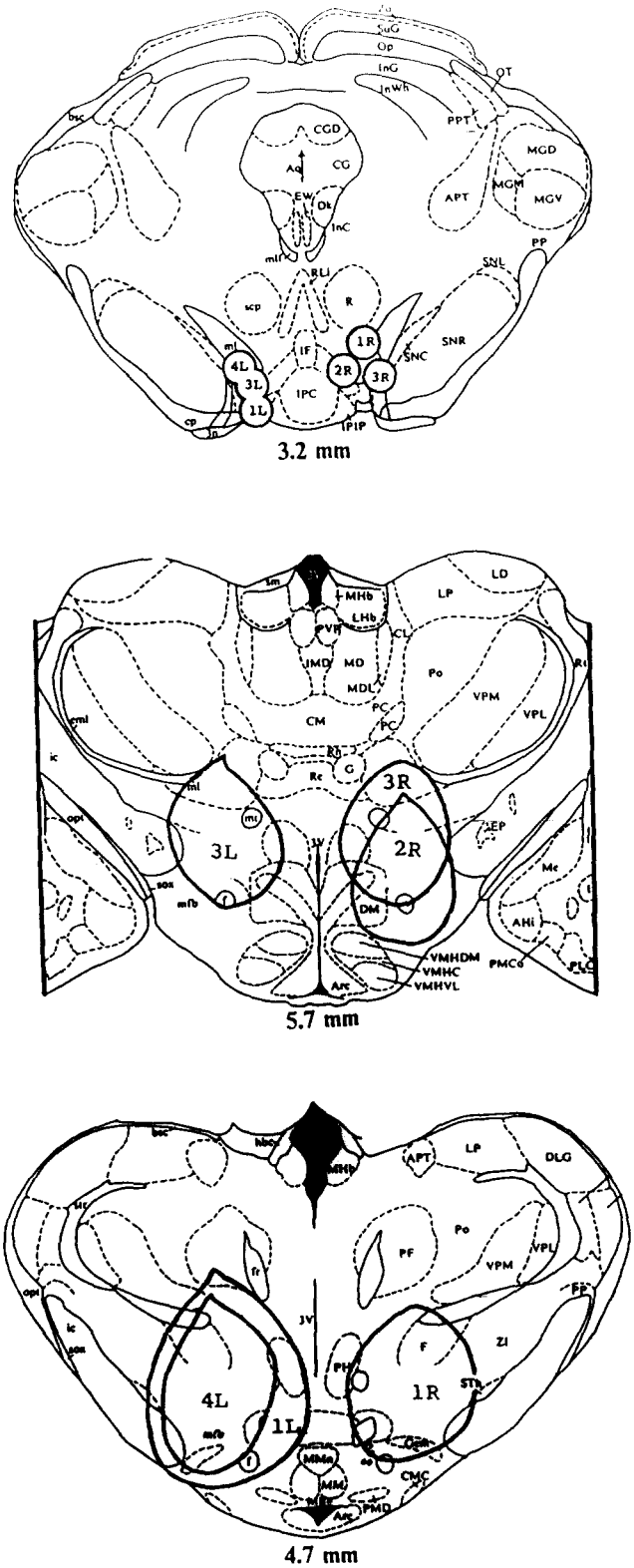

FIG. 4. Top panel: sites in the ventromedial midbrain which supported locomotor steiping before and after lesions of the mfb. Lower panels: illustrations of lesions of the mfb that were without significant effect on stepping elicited by VTA stimulation at sites indicated in the upper panel. The numbers indicate corresponding sites in the two figures. For example, stimulation site 4L in the top panel was ipsilateral to lesion 4L in the bottom panel.

mfb stimulation suggests mediation by a descending system. The alternative possibility of a necessary ascending pathway was not supported. Some role for an ascending pathway appeared feasible because the common area of the effective lesions was also the location of cell bodies of the dopaminergic system which ascend in the mfb [7] and appear to participate in locomotor control [3, 6, 10, 16]. If the stepping produced by mfb stimulation was due to activation of these or any ascending fibers from the midbrain, then lesions in the VTA might have blocked the response by removing the support of the ascending fibers provided by their caudally located cell bodies. However, the failure of large lesions of the mfb to block the stepping produced by stimulation of the VTA made this interpretation appear unlikely. Such lesions would be expected to at least extensively damage, if not totally interrupt, the ascending dopaminergic fibers from the VTA [7]. While these findings indicate that the ascending pathways are not essential for mfb stimulation to elicit stepping, they in no way discount the possibility of an important role for the ascending systems in normal locomotion.

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