Induction of Rhythmic Jaw Movements by Stimulation of the Mesencephalic Reticular Formation in the Guinea Pig

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This study was designed to investigate whether stimulation of the mesencephalic reticular formation (MRF) induces rhythmic jaw movements (RJMs) and, if it does so, to determine the RJM-inducing region in the MRF in ketamine-anesthetized guinea pigs. The results were as follows: (1) Repetitive electrical stimulation of the MRF at the level of the red nucleus induced rhythmic EMG bursts in the anterior digastric muscle (DIG). (2) The duration and cycle time of the rhythmic DIG EMG burst induced from the medial MRF were longer than those induced from the lateral MRF. (3) Repetitive MRF stimulation after paralysis still induced rhythmic multiple-unit activities in the anterior digastric motoneuron pool. (4) Neither precollicular decerebration nor cerebellectomy affected the MRF induction of RJMs. (5) Transverse hemisection at the rostral border of the pons abolished the RJMs induced from the contralateral, but not ipsilateral, MRF. Midline section of the midbrain abolished RJMs induced from the MRF on either side. (6) A lesion in the pontine pyramidal tract abolished the RJMs induced by stimulation of the ipsilateral cortical masticatory area (CMA), but not those induced from the contralateral MRF. (7) A unilateral lesion of the oral portion of the gigantocellular reticular nucleus, where the rhythm generator for the CMA-induced RJMs is located, abolished RJMs induced from not only the CMA, but also MRF on the contralateral side. (8) Microinjection of L-glutamate into the lateral, but not medial, MRF induced RJMs similar to those elicited by repetitive electrical stimulation of the same site. The results indicate that activation of MRF neurons can centrally induce RJMs via an extrapyramidal route, crossing to the contralateral side at the midbrain level and descending to the bulbar reticular formation.

Stimulation of various subcortical structures, as well as the cerebral cortex in a number of species, induces rhythmic jaw movements (RJMs), and the cortical area that induces RJMs upon stimulation has been called the cortical masticatory area (CMA) (see Luschei and Goldberg, 1981, for review). Lund and Dellow (1971) reported that RJMs could be induced by repet-itive electrical stimulation of an extensive subcortical region in rabbits, including the basal ganglia, corticobulbar tract, limbic structures, thalamus, and mesencephalic reticular formation (MRF). Sumi (1971) found, however, that repetitive electrical stimulation of the MRF not only enhanced, but also suppressed the cortically induced RJMs in rabbits. Recently, Chandler and Tal (1987) reported that repetitive microstimulation of widespread areas of the rostral pons and midbrain produced suppression of the cortically induced RJMs in guinea pigs. In addition, Tal (1987) demonstrated that the induction of RJMs by stimulation of the ventral MRF was, in fact, due to current spread to the fibers in the cerebral peduncle which carried the impulses from the CMA to the masticatory rhythm generator in the bulbar reticular formation of the guinea pig (Nozaki et al., 1986a).

The purpose of this study was (1) to determine whether electrical stimulation of the MRF can induce RJMs; (2) if it does, to delineate the exact location of the effective sites; and (3) to make clear whether activation of the MRF neurons is involved in the RJM induction by electrical stimulation of the MRF. A part of this study was reported in abstract form (Hashimoto et al., 1987).

Materials and Methods

Sixty-five female albino guinea pigs were used. Thirty minutes after they were given chlorpromazine hydrochloride (2.5 mg/kg, i.m.) and atropine sulfate (0.1 mg/kg, i.p.), ketamine hydrochloride was given (100 mg/kg, i.m.). A midline skin incision was made from the mandibular symphysis to the top of the sternum. After the trachea was cannulated, artificial ventilation was started. The masseter (MASS) and anterior digastric muscles (DIG) were exposed bilaterally. A pair of steel needle electrodes (0.2 mm diameter, enamel-coated except for 0.5 mm at the tip) were inserted into each of these muscles for recording EMG. After lidocaine was injected to the pressure points, the head of the animal was fixed to a stereotaxic apparatus after Rössner's method (1965; the interaural line: APO, HO). The spinal cord was transected at the C2 level. Parts of the frontal, temporal, and occipital bones were removed, and the dura was opened to expose the dorsal aspect of the cerebral cortex. The surface of the exposed cortex was covered with cotton wicks soaked with the physiological saline. An Ag–AgCl plate was placed subcutaneously in the left neck region. Anesthesia was maintained throughout the experiment by supplemental ketamine hydrochloride (10 mg/kg/hr, i.m.), except for 7 animals decerebrated at the precollicular level (stereotaxic coordinate A8.0), with a small knife made from a razor blade and mounted on an electrode carrier through a metal tube glued to its top.

The cerebellum was removed by suction in 11 animals. Midline sections of the brain stem at the midbrain level (3 animals), as well as a hemisection at the rostral border of the pons (8 animals, including one decerebrate and one cerebellectomized animal) were performed by means of the same type of small knife as used for decerebration. The pyramidal tract was sectioned at the rostral pontine level (A2.0) unilaterally in 4 animals, including 1 decerebrate and 1 cerebellectomized animal, on the side contralateral to stimulation of the MRF, by passing positive

direct current (90 μA for 30 sec) through tungsten electrodes (tip resistance, 0.5–1 MΩ). A localized electrolytic lesion was made unilaterally in the bulbar reticular formation of 4 animals by passing positive direct current (90 μA, 30 sec) through a tungsten electrode (tip resistance, 3–5 MΩ) stereotaxically inserted into the oral portion of the gigantocellular reticular nucleus (P2.0–2.2, L1.5–2.0, HO), where repetitive stimulation of the contralateral cortical masticatory area (CMA) induced a rhythmic field potential in the masticatory rhythm (Nozaki et al., 1986a). These lesions of the brain stem and the pyramidal tract were made under insufflation of 0.3% halothane. Body temperature was kept at about 37°C with a heating pad under the body and an incandescent lamp from above.

An electropolished, enamel-coated tungsten microelectrode (tip resistance, 0.5–1.0 MΩ) was inserted vertically into the CMA (stereotaxic coordinates A15.5–16.0, L4.0) to a depth of 2.0 mm on one side. Repetitive electrical stimulation (40 Hz, 0.3 msec, 100–350 μA) was delivered through this electrode, which was fixed to the point where the threshold for induction of RJMs was lowest. Another tungsten microelectrode of the same type was inserted vertically into the brain stem for stimulation. To study the sites effective for inducing RJMs, repetitive pulses at 40 Hz were systematically applied every 500 μm in the rostrocaudal and mediolateral directions and 250 μm in the vertical direction. The explored region extended from A3.0 to A8.0, from L0 to L3.5, and from the dorsal to ventral surface of the brain stem.

After the animal was paralyzed with pancuronium bromide (1.2 mg/kg, i.m.), the induced rhythm was monitored by the multiple unit activity (MUA) in the anterior digastric motoneuron pool on the side contralateral to the cortical and brain-stem stimulations. The electrode (tip resistance, 5–8 MΩ) was fixed to the point where the largest antidromic field potential was recorded after stimulation of the anterior digastric nerve with the electrode for recording DIG EMG.

The EMG, MUA, and field potentials were displayed on an oscilloscope after amplification (bandwidths: 33–3000 Hz, for EMG, 150–3000 Hz for MUA, and 0.08–3000 Hz for field potential) and recorded on an FM tape recorder (flat frequency response from DC to 2500 Hz) for later analysis. Photographic and ink-written records were made from the recorded data. RJMs were videotaped in some experiments; the distance between the upper and lower incisal edges (interincisal distance) was measured at the largest open position during the jaw movement to monitor the maximal displacement of the jaw during each jaw-opening.

Glutamate (L-glutamic acid, monosodium) dissolved in 0.9% saline (50 mM, pH 8.0) was injected into the brain stem in 7 animals with a Hamilton microsyringe mounted on an electrode carrier. The solution also contained methylene blue (0.01 gm/ml) to mark the injection site. The injection needle was initially lowered stereotaxically to the dorsolateral part of the MRF; it was then left in place for 10 min to allow stabilization of the tissue. Subsequently, 4.0 μl glutamate solution was slowly injected over a period of 3 min. The injection needle was left in place for at least 10 min after injection. As a control, the same amount of the physiological saline was injected into the same region after the same procedure.

At the termination of each experiment, an overdose of pentobarbital sodium was given intraperitoneally, and microlesions were made at the stimulated and recorded points in the brain stem by applying positive DC (70 μA, 15 sec) through stimulating and recording electrodes. The brain was taken out and kept in 10% formalin solution for 1 week. Serial coronal or horizontal frozen sections, 75 μm thick, were made of the brain stem, stained with cresyl violet for verification of the stimulation and recording sites.

Results

RJMs and jaw muscle EMGs induced by repetitive electrical stimulation of the mesencephalic reticular formation

Tungsten stimulating electrodes were stereotaxically inserted into the MRF on one side vertically through the superior colliculus. Repetitive electrical stimulation of the MRF induced rhythmic opening and closing movements of the jaw. The RJM was similar to that induced by repetitive electrical stimulation of the CMA in that it was accompanied by a lateral movement to the side contralateral to stimulation. The lateral displacement of the jaw was larger in the opening movement induced from the lateral MRF (L2.0–3.0) than that from the CMA, but there

was no apparent difference in the lateral displacement of the jaw between that induced from the medial MRF (L0.5–1.5) and that induced from the CMA. The vertical displacement of the jaw during the opening movement induced from the MRF, either medial or lateral (interincisal distance, 7–8 mm), far exceeded that induced from the CMA (interincisal distance, 3–4 mm). The RJM induced from the medial MRF was accompanied by a rhythmic protrusion and retraction of the tongue in coordination with the RJMs, while that induced from the lateral MRF was accompanied by essentially no tongue movements.

Figure 1 illustrates the rhythmic EMG burst activities of the anterior DIG and MASS muscles on both sides induced from the lateral (Fig. 1B) and medial (Fig. 1C) MRF on the left side compared with those induced from the left CMA (Fig. 1A) in an animal.

To compare the pattern of RJMs induced from these 3 different loci, samples of 10 consecutive DIG EMG cycles were obtained during a steady period of the induced RJMs in 5 animals. The cycle time and burst duration of the rhythmic DIG EMG were measured and averaged with respect to each RJM sequence. The values in Table 1 represent the mean and SD of these averaged cycle time and burst durations of each RJM sequence.

The left column of Table 1 shows the cycle time of the rhythmic DIG EMG burst induced by repetitive stimulation of the CMA and lateral and medial MRF. The cycle time of the RJM induced from the medial MRF was longer than that from the CMA ($p < 0.05$, t test), while that induced from the lateral MRF was slightly, but significantly, shorter than that from the CMA ($p < 0.05$, t test).

The rhythmic burst activity appeared in the homonymous muscles on both sides, whether it was induced by stimulation of the CMA or MRF. The rhythmic EMG activity of the DIG was virtually the reciprocal of that of the MASS when induced by the CMA stimulation (Fig. 1A). When induced by stimulation of the lateral MRF (Fig. 1B), however, the rhythmic burst activity of the MASS EMG occurred both in and out of phase with the DIG EMG activity. The out-of-phase burst appeared directly following the in-phase burst at the early interburst phase of the DIG EMG burst with a shorter duration and a higher amplitude than the in-phase burst. On the other hand, stimulation of the medial MRF induced very little MASS EMG activity (Fig. 1C).

The right column of Table 1 shows the duration of the rhythmic DIG EMG burst induced by repetitive stimulation of the CMA and lateral and medial MRF. The duration of DIG EMG bursts induced from the medial MRF was longer than that induced from either the CMA or lateral MRF ($p < 0.05$, t test), while that induced from the lateral MRF was slightly shorter than that induced from the CMA ($p < 0.05$, t test). When the electrode track was moved mediolaterally or lateromedially, the change from one to the other of the above-mentioned 2 patterns induced from the lateral and medial MRF occurred gradually rather than abruptly: when we moved the points of insertion from the lateral to medial portion, the cycle time gradually lengthened, and this was accompanied by a gradual decrease in amplitude of the MASS EMG burst.

Effects of paralysis on the centrally induced rhythmic activity of the anterior digastric motoneurons

Repetitive electrical stimulation of the MRF induced a rhythmic MUA in the anterior digastric motoneuron pool (Fig. 2, *Aa, Ba,*
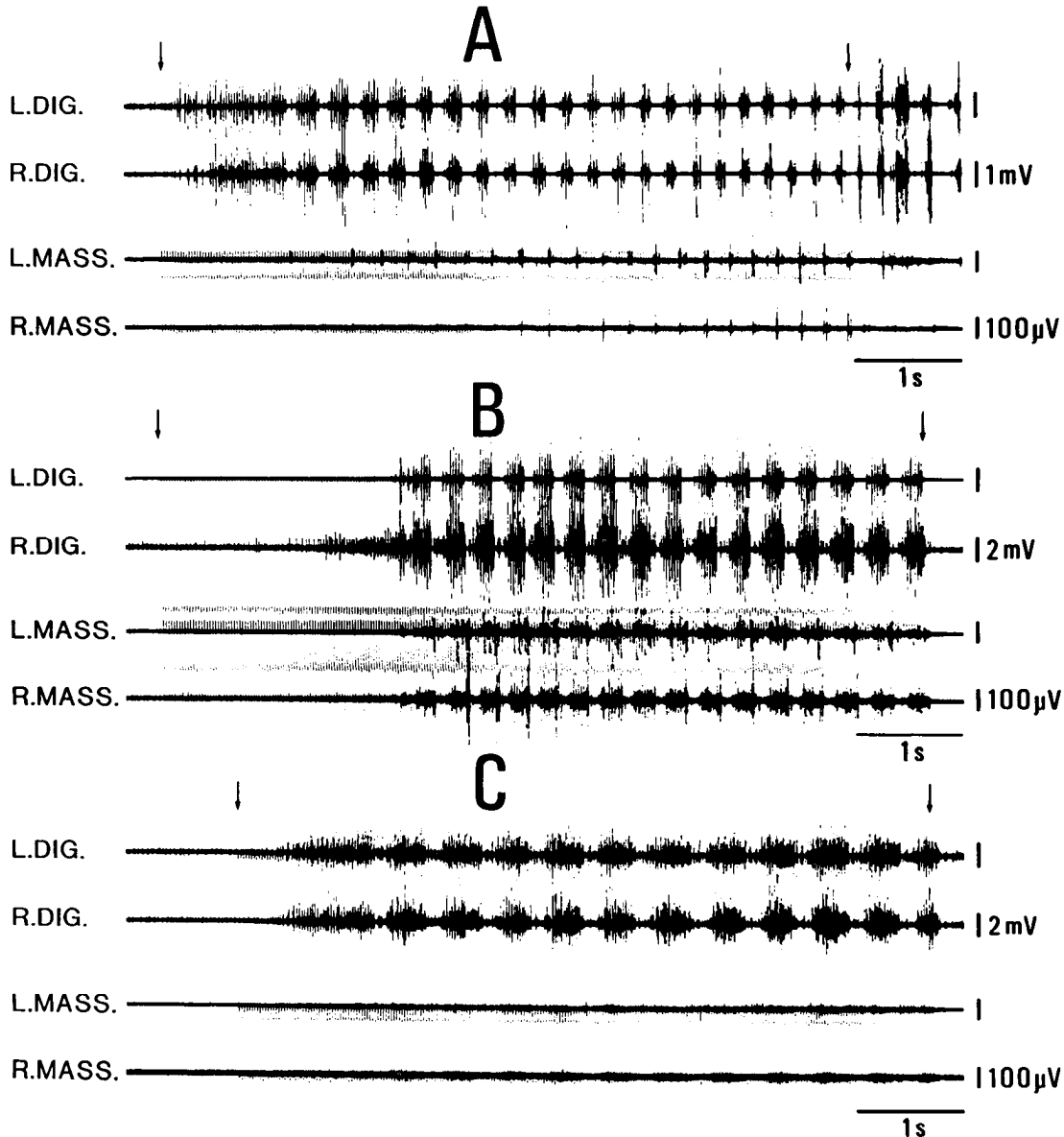
Figure 1. Rhythmic jaw muscle EMG activities induced by repetitive electrical stimulation of the cortical masticatory area (CMA) and mesencephalic reticular formation (MRF). *A,* Stimulation of the left CMA (40 Hz, 0.3 msec, 150 μA). *B,* Stimulation of the left lateral MRF (40 Hz, 0.3 msec, 150 μA). *C,* Stimulation of the left medial MRF (40 Hz, 0.3 msec, 150 μA). *Left* and *right arrows* represent the time of onset and cessation of stimulation, respectively. Abbreviations (common to this and the following figures): *DIG,* anterior digastric muscle; *MASS,* masseter muscle; *L,* left; *R,* right. Voltage calibration: 1 mV (*A*) or 2 mV (*B, C*) for DIG; 100 μV for MASS. Time base: 1 sec, common to *A–C. A–C* were obtained from an animal.

lower traces) corresponding in time with the DIG EMG (Fig. 2, *Aa, Ba,* upper traces). This rhythmic MUA was still induced after the animal was paralyzed with essentially the same pattern as before paralysis, although the cycle time tended to be slightly longer after paralysis (Fig. 2, *Ab, Bb*).

Effects of decerebration and cerebellectomy on the rhythmic DIG EMG activity induced by the MRF stimulation

The rhythmic digastric EMG burst induced by stimulation of the lateral (Fig. 3*A,* left panel) and medial MRF (Fig. 3*B,* left panel) persisted after transection of the brain stem at the precollicular level (A8.0) in all the 7 animals tested (Fig. 3, *A, B,* right panels). Likewise, cerebellectomy did not abolish the MRF-

induced rhythmic burst of the DIG EMG in any of 11 decerebellated animals (Fig. 4).

Effects of hemisection of the brain stem at the rostral border of the pons on the MRF-induced rhythmic DIG EMG activity

To study whether the route responsible for MRF induction of the rhythmic DIG EMG activity descends ipsi-, contra-, or bilaterally in the caudal brain stem, a unilateral transection was performed at the rostral border of the pons. Figure 5 illustrates the effects of hemisection of the brain stem on the MRF-induced RJMs in an animal. Electrical stimulation of the MRF on the left (Fig. 5*A,* left panel), as well as right MRF (Fig. 5*B,* left panel), induced a rhythmic burst in the DIG EMG on both
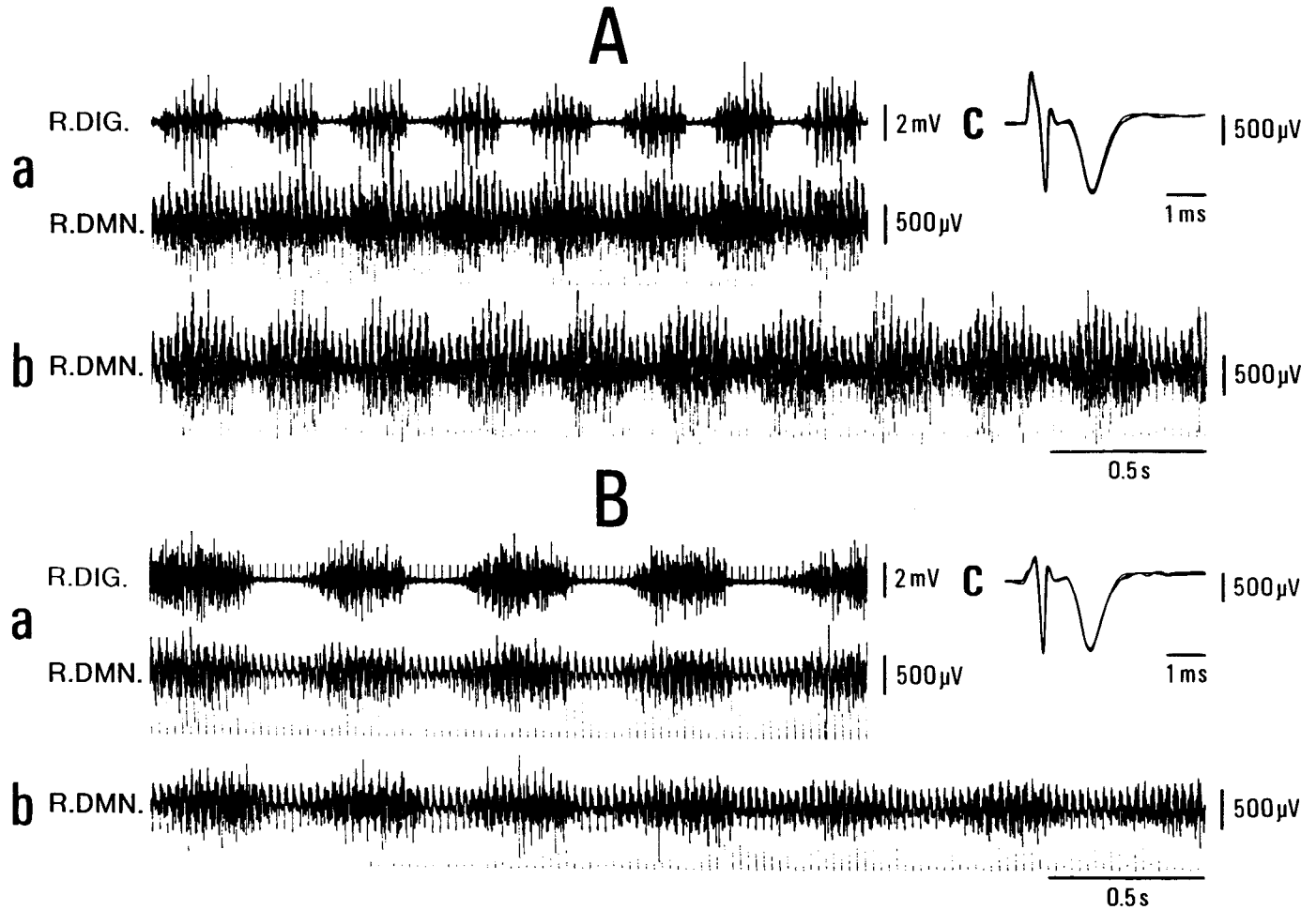
Figure 2. Effects of paralysis on rhythmic multiple-unit activity (MUA) of anterior digastric motoneurons induced by repetitive electrical stimulation of the MRF. *Aa* and *b*, Stimulation of the left lateral MRF (40 Hz, 0.3 msec, 150 μA). *Ba* and *b*, Stimulation of the left medial MRF (40 Hz, 0.3 msec, 150 μA). *a*, DIG EMG (*upper trace*) and MUA of anterior digastric motoneurons (*DMNs, lower trace*), which was recorded from the anterior digastric motoneuron pool in the right trigeminal motor nucleus, where stimulation (1 mA, 0.1 msec) of the right anterior digastric nerve evoked the maximum antidromic negative field potentials (*Ac, Bc*); *b*, MUA of DMNs. *Aa* and *Ba*, Before paralysis, *Ab* and *Bb*, after paralysis. *A* and *B* were obtained from different animals.

sides. After a hemisection was made on the left (Fig. 5*C*), the rhythmic DIG EMG burst was still induced by stimulation of the MRF on the left side (Fig. 5*A*, right panel) but not from the right side (Fig. 5*B*, right panel). The same results were obtained in all the 8 animals tested, which indicates that the route crossing to the contralateral side at the midbrain level can by itself induce

Table 1. Cycle time and burst duration of rhythmic DIG EMG activity

	CT (msec ± SD)	BD (msec ± SD)
CMA (*n* = 31)	303 ± 59	264 ± 64
L.MRF (*n* = 18)	269 ± 46	226 ± 48
M.MRF (*n* = 8)	602 ± 199	528 ± 192

CT and BD represent the mean and SD of the averaged cycle time and burst duration of each rhythmic 10 consecutive DIG EMG sequence induced by repetitive central stimulations (40 Hz, 0.3 msec, 1.3 times the threshold intensity). CT, cycle time; BD, burst duration; L, lateral; M, medial. *n* represents the number of measured RJM sequences, each consisting of 10 consecutive DIG EMG sequences.

rhythmic DIG EMG activity, but uncrossing descending routes, if any, cannot.

Effects of midline section of the brain stem at the midbrain level on the rhythmic DIG EMG activity induced by the MRF stimulation

To get further evidence for crossing of the route responsible for the MRF induction of RJMs at the midbrain level, we studied the effects of a midline section of the brain stem from the rostral pole of the superior colliculus to the intercollicular level (corresponding to the rostral and caudal borders of the midbrain, respectively) on the MRF induction of RJMs in 3 animals. A midline section abolished RJMs induced by stimulation of the MRF on either side in all the 3 animals tested. Left panels of Figure 6, *A, B*, shows the bilateral, rhythmic DIG EMG burst activity induced by stimulation of the left and right MRF in an animal, respectively. After a midline section was made at the midbrain level, stimulation of either the left (Fig. 6*A*, right panel) or right (Fig. 6*B*, right panel) MRF no longer induced RJMs. The results indicate that the route crossing at the midbrain level is solely responsible for induction of RJMs by MRF stimulation.
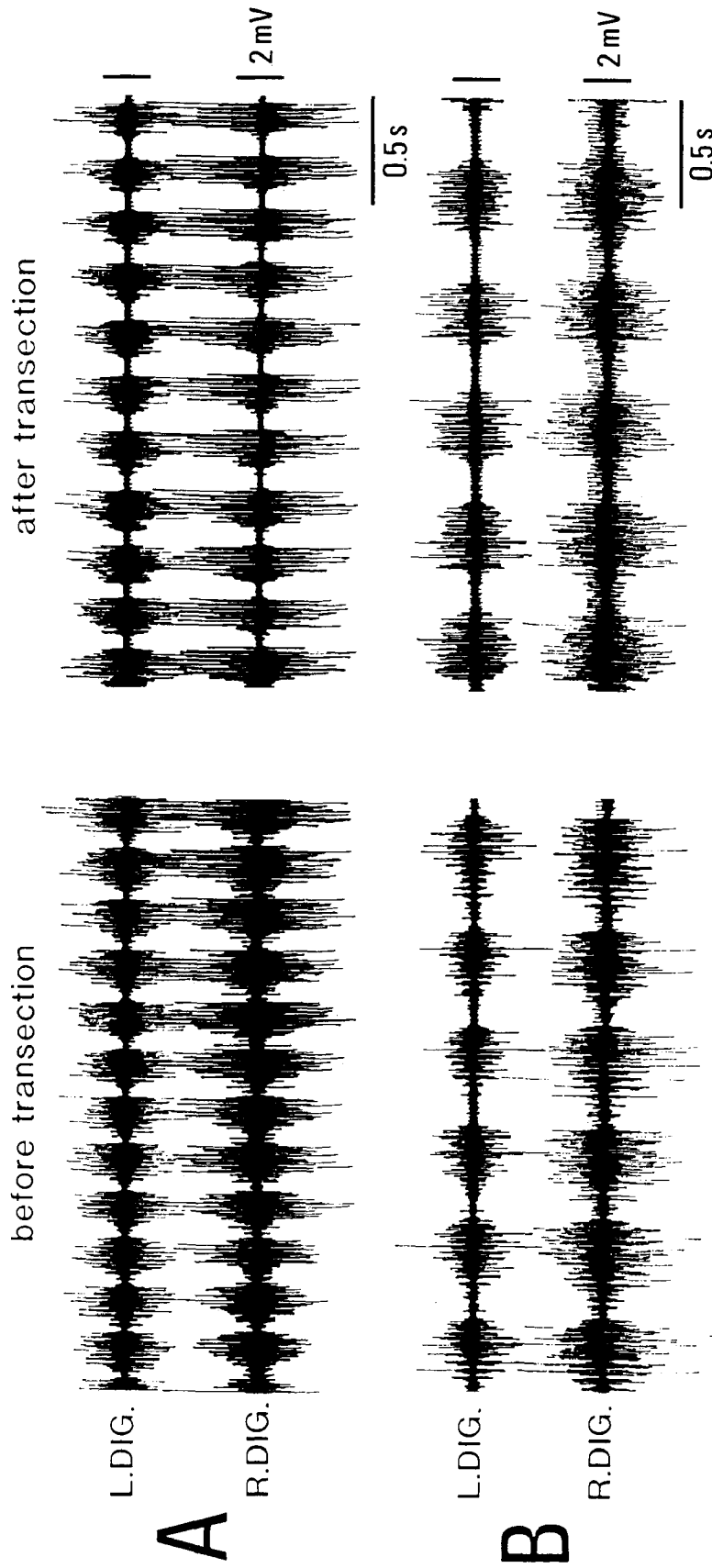
Figure 3. Effects of precollicular decerebration on MRF-induced rhythmic DIG EMG activity. *A* and *B* were obtained during repetitive stimulation (40 Hz, 0.3 msec, 180 μA) of the lateral (*A*) and medial (*B*) MRF on the left side before (*left panels*) and after (*right panels*) decerebration in an animal. Voltage and time calibrations in the *right panel* also apply to the *left.*
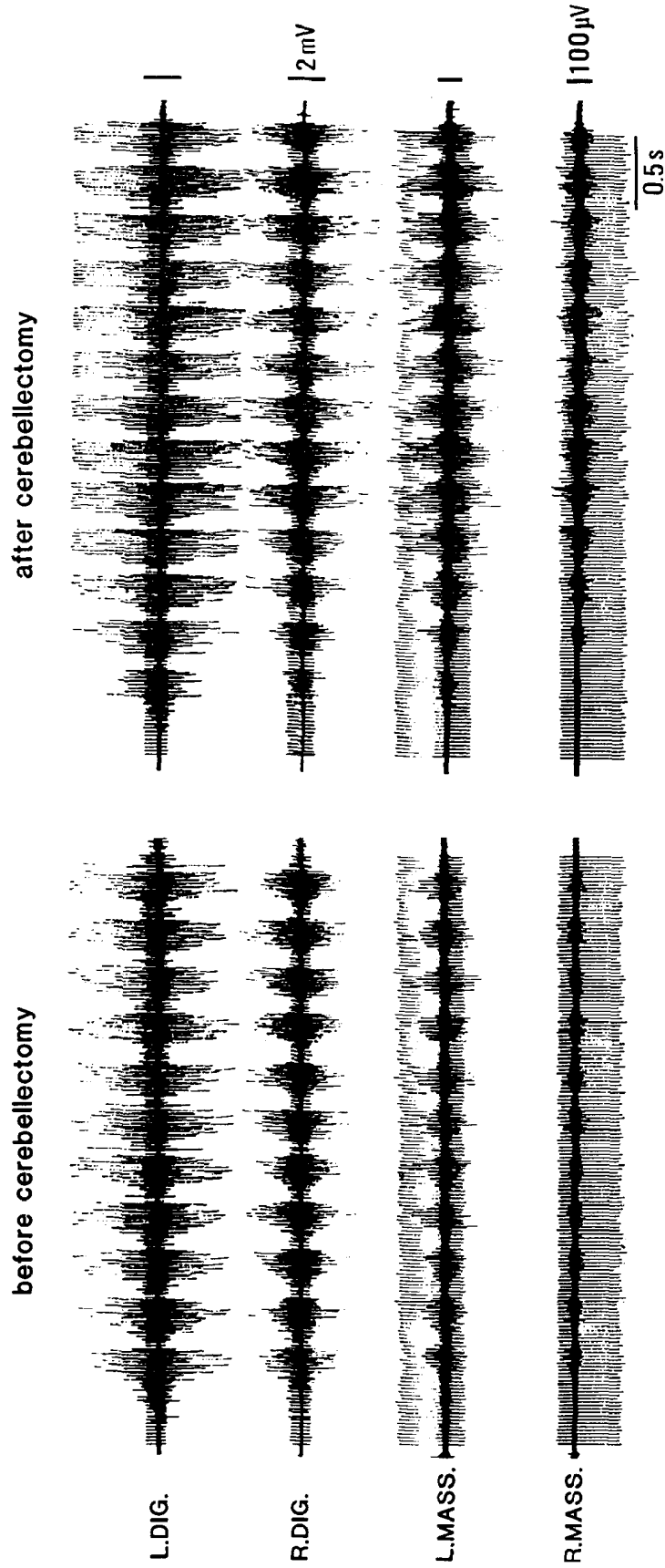
Figure 4. Effects of cerebellectomy on MRF-induced rhythmic jaw muscle EMG activity. Repetitive electrical stimulation (40 Hz, 0.3 msec, 180 μA) was applied to the right lateral MRF before (*left panel*) and after (*right panel*) cerebellectomy in an animal. Voltage and time calibrations in the *right* panel also apply to the *left*.
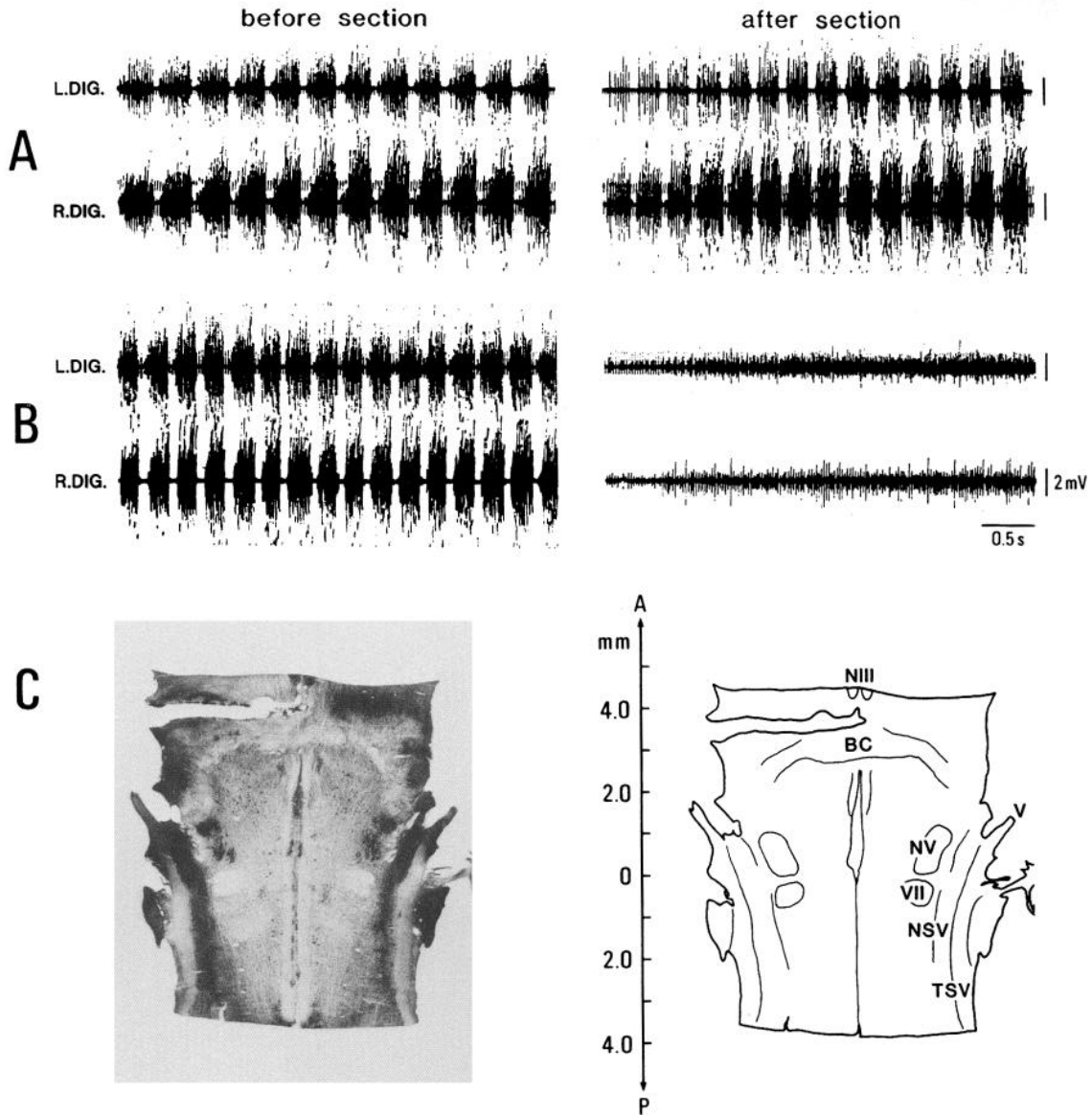
Figure 5. Effects of a hemisection of the brain stem on MRF-induced rhythmic DIG EMG activity. *A* and *B* were obtained during repetitive electrical stimulation (40 Hz, 0.3 msec, 200 μA) of the lateral MRF on the left (*A*) and right (*B*) sides, respectively, before (*left panels*) and after (*right panels*) hemisection at the rostral border of the pons in an animal. *Vertical bars* on the *right* panels represent voltage calibration of 2 mV and a *horizontal bar* represents time calibration of 0.5 sec; they are common to *left panels*. *C,* Photomicrograph (*left*) and schematic drawing (*right*) of horizontal histological section showing the site of hemisection on the left side. Abbreviations: *BC,* brachium conjunctivum; *NIII,* oculomotor nucleus; *NSV,* trigeminal spinal nucleus; *NV,* trigeminal motor nucleus; *TSV,* trigeminal spinal tract; *V,* trigeminal nerve; *VII,* facial nerve.

Effects of pontine pyramidal tract lesions on the MRF-induced rhythmic DIG EMG activity

Impulses inducing RJMs from the CMA were reported to descend solely in the corticobulbar projection fibers (Nozaki et al., 1986b). To check whether the MRF-induced RJM was due to current spread to these corticobulbar fibers, we made a lesion in the pyramidal tract at the pontine level, i.e., caudal to the MRF stimulation site, in 4 animals. Figure 7 illustrates the results obtained from an animal in which a pyramidal tractotomy was done on the right side. Before making this lesion, repetitive stimulation of the lateral (Fig. 7*B*, left panel) and medial MRF (Fig. 7*C*, left panel) on the left side, as well as the CMA on the right side (Fig. 7*A*, left panel), induced rhythmic

activity in the DIG EMG. After making a lesion in the right pyramidal tract (Fig. 7*D*), the rhythmic DIG EMG activity was no longer induced by stimulation of the right CMA (Fig. 7*A*, right panel). In contrast, stimulation of both the lateral (Fig. 7*B*, right panel) and medial MRF (Fig. 7*C*, right panel) on the left side still induced the rhythmic DIG EMG burst. The EMG pattern was essentially the same as that before making the lesion (Fig. 7, *B, C,* left panels).

Effects of bulbar reticular formation lesions on MRF-induction of RJM

It was reported that the central rhythm generator responsible for induction of the rhythmic masticatory jaw movement by stimulation of the CMA of the guinea pig was located in the
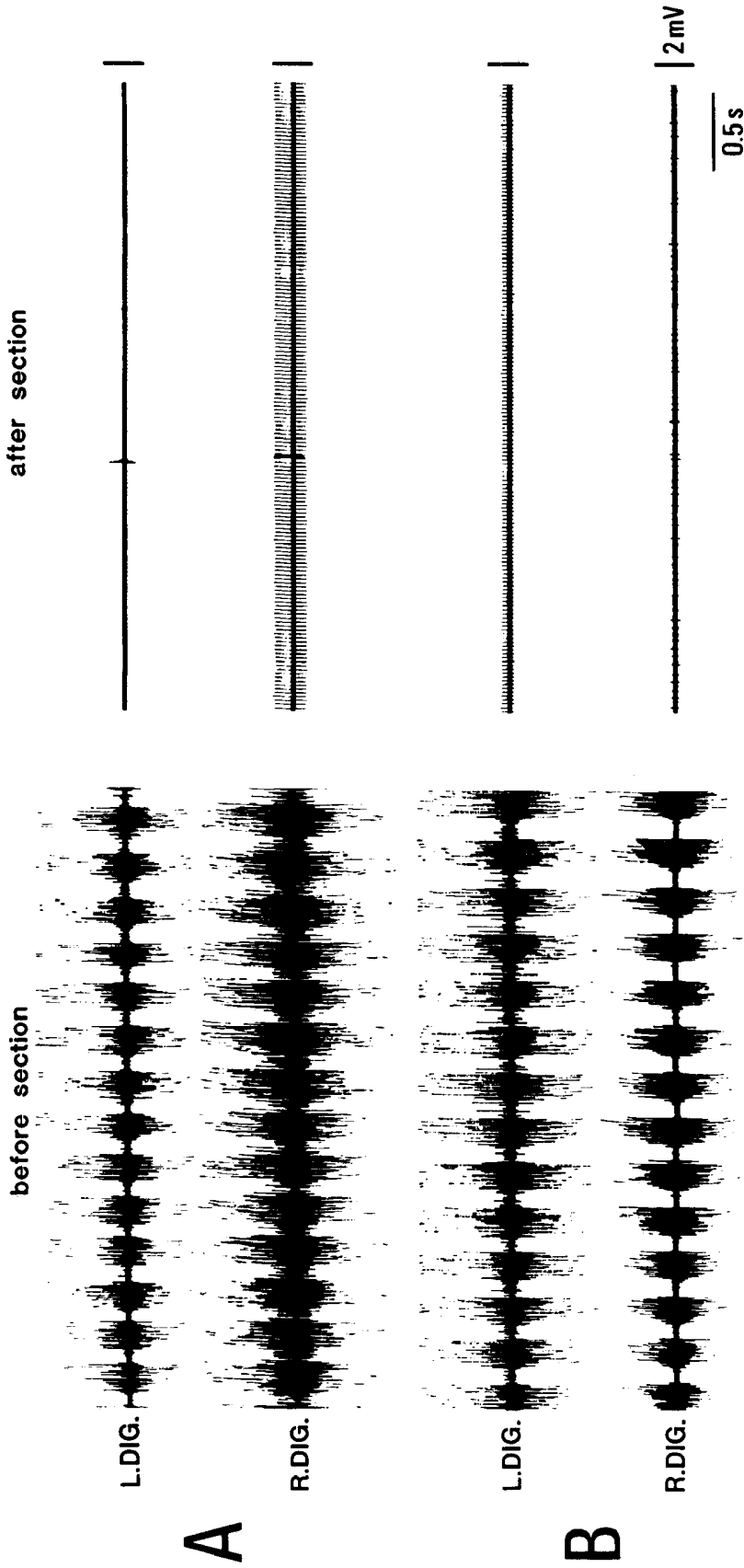
Figure 6. Effects of a midline section of the brain stem at the midbrain level on MRF-induced rhythmic DIG EMG activity. *A* and *B* were obtained during repetitive electrical stimulation (40 Hz, 0.3 msec, 170 μA) of the lateral MRF on the left (*A*) and right (*B*) sides, respectively, before (*left panels*) and after (*right panels*) the midline section of the brain stem from the rostral pole of the superior colliculus to the intercollicular level in an animal. *Vertical bars* on the *right panels* represent voltage calibration of 2 mV and a *horizontal bar* represents time calibration of 0.5 sec; they also apply to the *left panels*.
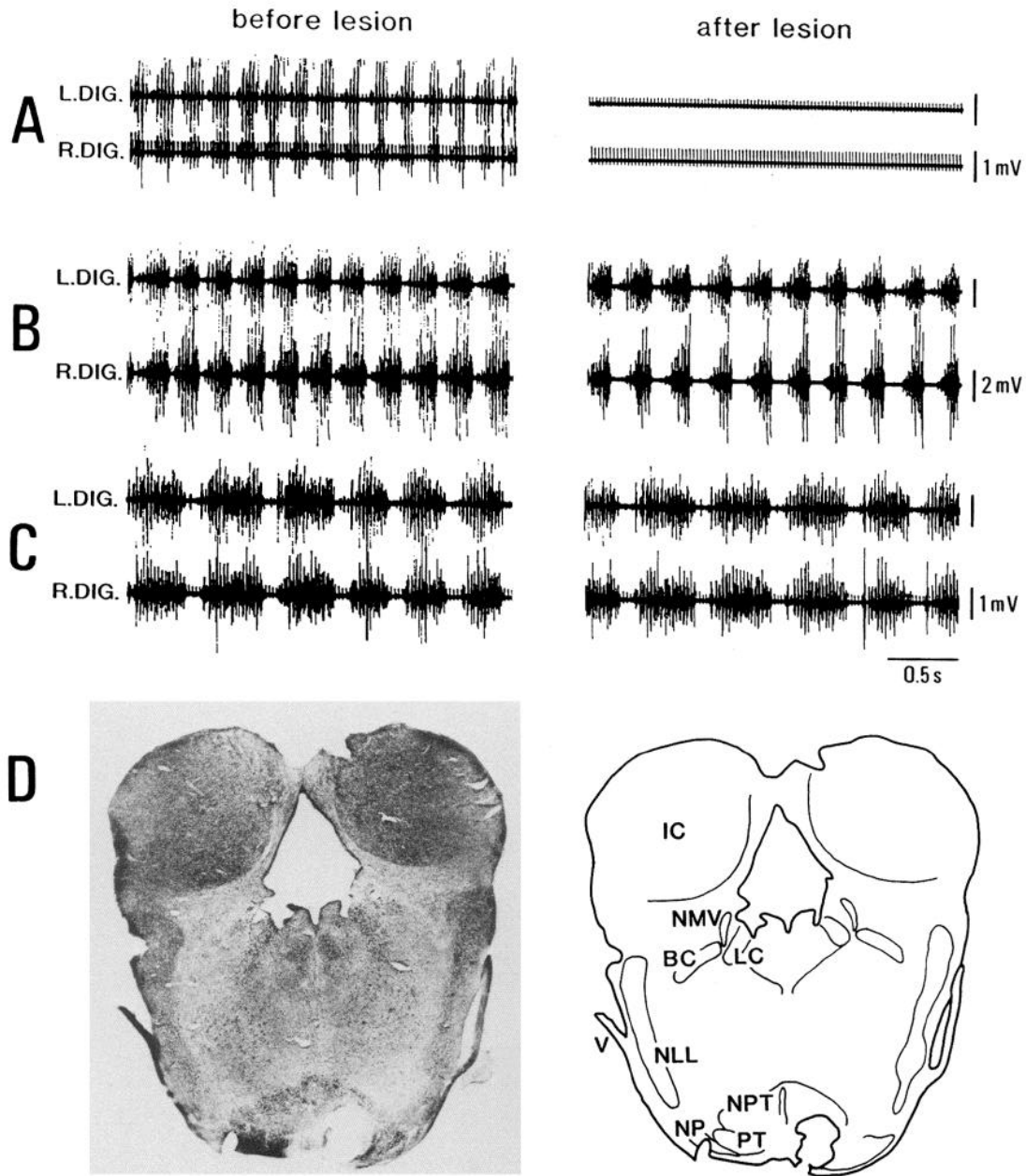
Figure 7. Effects of unilateral lesion of the pontine pyramidal tract on the rhythmic DIG EMG activity induced by stimulation of the CMA and MRF. *A,* CMA stimulation (40 Hz, 0.3 msec, 180 μA) on the right side. *B,* Stimulation (40 Hz, 0.3 msec, 200 μA) of the lateral MRF on the left side. *C,* Stimulation (40 Hz, 0.3 msec, 180 μA) of the medial MRF on the left side. *Left* and *right panels* of *A–C* were obtained during repetitive stimulation in an animal before and after lesions of the pyramidal tract, respectively. *D,* Photomicrograph (*left*) and schematic drawing (*right*) of a frontal histological section showing the lesion of the right pyramidal tract. *Vertical bars* at right represent calibration of 1, 2, and 1 mV for *A, B,* and *C,* respectively. Time base: 0.5 sec, common to *A–C.* Abbreviations: *IC,* inferior colliculus; *LC,* locus coeruleus; *NMV,* trigeminal mesencephalic nucleus; *NLL,* nucleus of lateral lemniscus; *NP,* nuclei pontis; *NPT,* nucleus reticularis tegmenti pontis; *PT,* pyramidal tract; others as in Figure 5.

oral portion of the gigantocellular reticular nucleus (GCo) in the medulla oblongata (Nozaki et al., 1986a). The left panels of Figure 8, *A, B,* shows the rhythmic MUA burst in the right anterior digastric motoneuron pool (upper trace) and the rhythmic field potential in the right GCo (lower trace) induced by repetitive electrical stimulation of the left CMA and MRF, respectively, in a paralyzed animal. After a lesion was made in the right GCo region (Fig. 8C), stimulation of either the CMA or MRF on the left side no longer induced the rhythmic MUA in the anterior digastric motoneuron pool (Fig. 8, *A, B,* right

panels). The same result was obtained from all the 3 other animals tested.

Induction of RJM by chemical stimulation of the MRF

To study whether activation of the MRF neurons in the stimulated site is involved in induction of RJMs by electrical stimulation of the MRF, glutamate (4.0 μl) was injected into the MRF of 7 animals. Injection of glutamate into the lateral MRF induced rhythmic EMG bursts similar to those induced by electrical stimulation of the lateral MRF in 5 out of 8 trials in 5
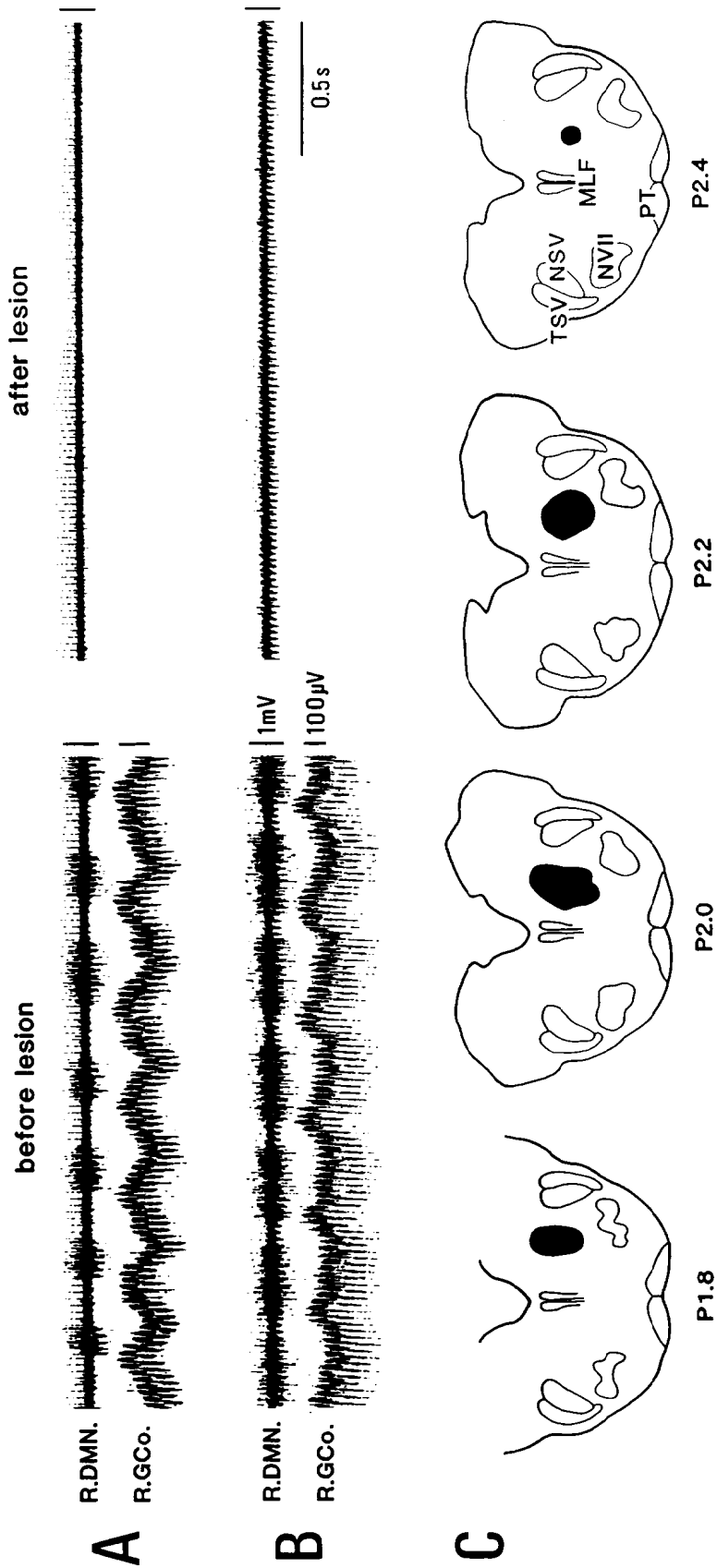
Figure 8. Effects of a lesion in the rostral portion of the gigantocellular reticular nucleus on the rhythmic MUA in the anterior digastric motoneuron pool induced by repetitive electrical stimulation of the CMA (40 Hz, 0.3 msec, 270 μA) and MRF (40 Hz, 0.3 msec, 180 μA) on the left side. *A* and *B: Left panels,* Simultaneous records of the rhythmical MUA in the right anterior digastric motoneuron pool (*R.DMN*) and the rhythmic field potential (negative is *down*) in the right gigantocellular reticular nucleus (oral portion; *R.GCo*) induced by repetitive electrical stimulation of the CMA (*A*) and lateral MRF (*B*) on the left side. *Right panels,* Responses recorded from the right anterior digastric motoneuron pool to the same stimulation of the left CMA (*A*) and lateral MRF (*B*) after making a lesion in the right GCo by application of positive direct current (90 μA, 30 sec) to 5 points through the electrode used for recording of the field potentials in *A* and *B. Vertical bars* at *right* of each panel show voltage calibration of 1 mV for R.DMN and 100 μV for R.GCo. Time base in the *right panel* of *B* is common to *A* and *B. C,* Schematic drawings of frontal histological sections of the brain stem showing the location of the lesion in the bulbar reticular formation. Alphanumerals represent the anteroposterior stereotaxic coordinates. Abbreviations: *MLF,* medial longitudinal fasciculus; *NVII,* facial nucleus; others, same as in Figure 5.
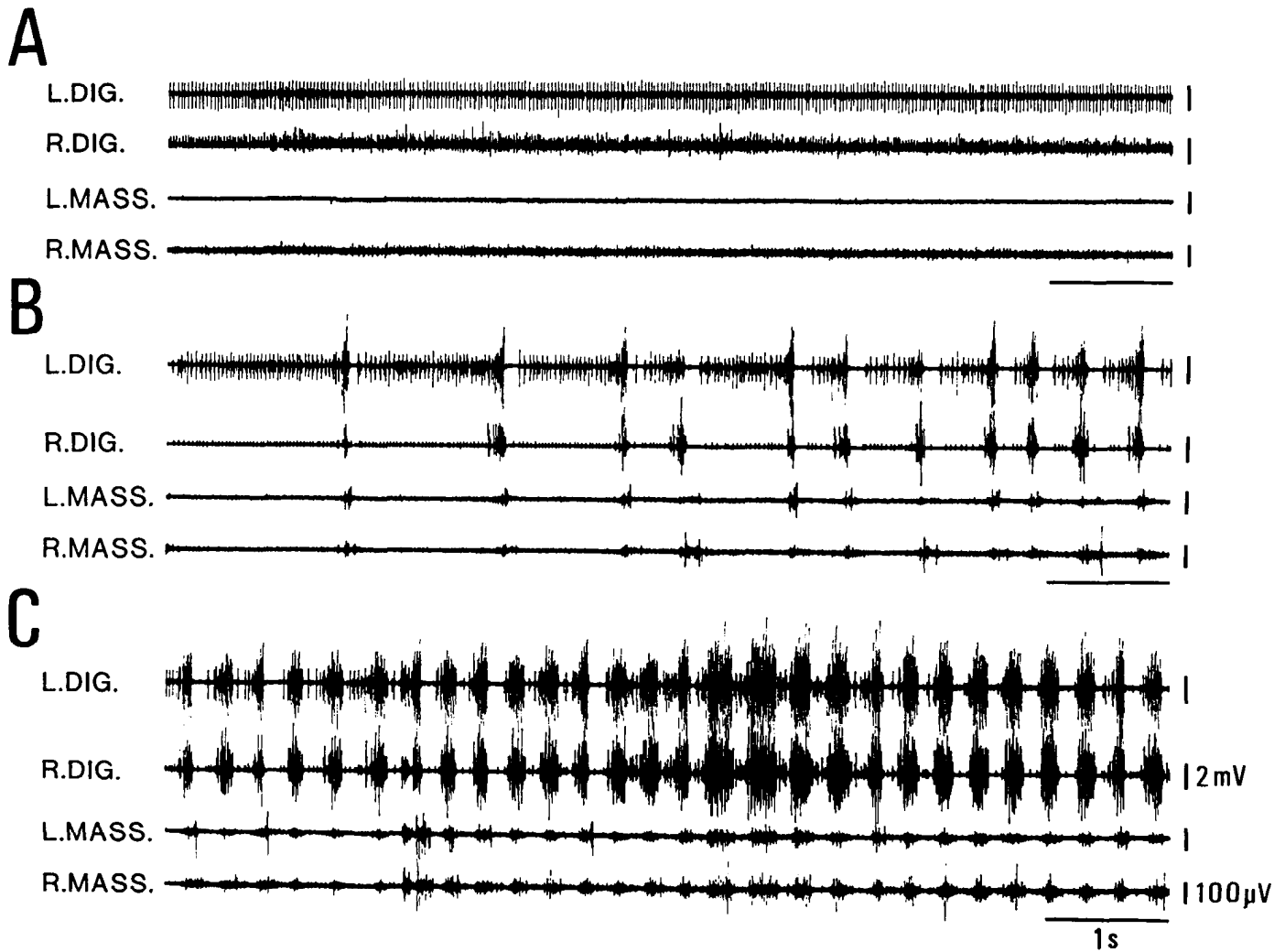
Figure 9. Rhythmic jaw-muscle EMG activities induced by chemical stimulation of the MRF. *A–C* were recorded about 1, 7, and 8 min after the onset of injection of L-glutamate (50 mM, 4.0 μl) into the right lateral MRF, respectively. *Vertical bars* at *right* in *A–C* represent voltage calibration of 2 mV for DIG and 100 μV for MASS; time bases in *A–C* represent 1 sec.

animals, while no rhythmic activity was induced in any of 3 trials in 2 animals in which glutamate was injected into the medial MRF. Injection of the same amount of glutamate into either the MRF more lateral than 3.0 mm from the midline (3 trials in an animal) or the superior colliculus (3 trials in 2 animals) did not induce RJMs.

Figure 9 illustrates the effects of this chemical stimulation of the lateral MRF (the injected point is shown by a square in Fig. 10) in an animal, in which no EMG activity was seen in either the DIG or MASS before injection of glutamate. About 30 sec after the onset of injection of glutamate into the right lateral MRF, tonic EMG activity appeared in both the DIG and MASS on both sides, which was more dominant in the DIG EMG (Fig. 9*A*), and it continued for about 6 min. About 7 min after the onset of injection, burst activities began to appear at irregular intervals (Fig. 9*B*, left portion). The interval gradually became shorter and regular (Fig. 9*B*, right portion). About 8 min after the onset of injection, the rhythmic EMG activity showed almost the same pattern as that induced by electrical stimulation of the lateral MRF. This regular rhythmic EMG activity persisted for about 20 min. Subsequently, the cycle time gradually

became longer and longer. About 40 min after the injection of the EMG activity became silent as before the injection. Injection of the same amount of saline into the same point in the lateral MRF induced no EMG activity in any jaw muscle.

Histological examination of the brain stem in 5 animals revealed that the infusion spread of methylene blue contained in 4.0 μl of injected glutamate solution was limited to an area approximately 1 mm in size from the injected points.

Sites in the midbrain inducing RJMs by electrical stimulation

Histological examination revealed that the RJM with a large opening movement was induced by repetitive electrical stimulation of the MRF surrounded by the superior colliculus, medial geniculate body, medial lemniscus, red nucleus, and central gray matter. In the rostrocaudal direction it was confined within ca. 1 mm at the level of the red nucleus. The region with low thresholds for RJM induction extended from the lateral to most medial part of the dorsal MRF, as schematically illustrated in Figure 10. Larger filled circles represent stimulated points with lower thresholds. No field potentials were evoked in this region

Figure 10. Schematic representation of MRF sites inducing RJMs, projected on a frontal section of the brain stem at A5.8. *Circles* show the points whose repetitive electrical stimulation (40 Hz, 0.3 msec) induced RJMs at intensities not more than 180 μA; their diameters are drawn in inverse proportion to the threshold for RJM induction, as shown at *bottom. Vertical* and *horizontal* scales correspond to dorsoventral and horizontal axes of the stereotaxic fixation employed in the present study. Abbreviations: *CG,* central gray matter; *D,* nucleus of Darkschewitsch; *IP,* interpeduncular nucleus; *LM,* medial lemniscus; *MG,* medial geniculate body; *NR,* red nucleus; *PED,* cerebral peduncle; *SC,* superior colliculus; *SN,* substantia nigra. The *square* represents of the site of glutamate injection, the results of which are shown in Figure 9.
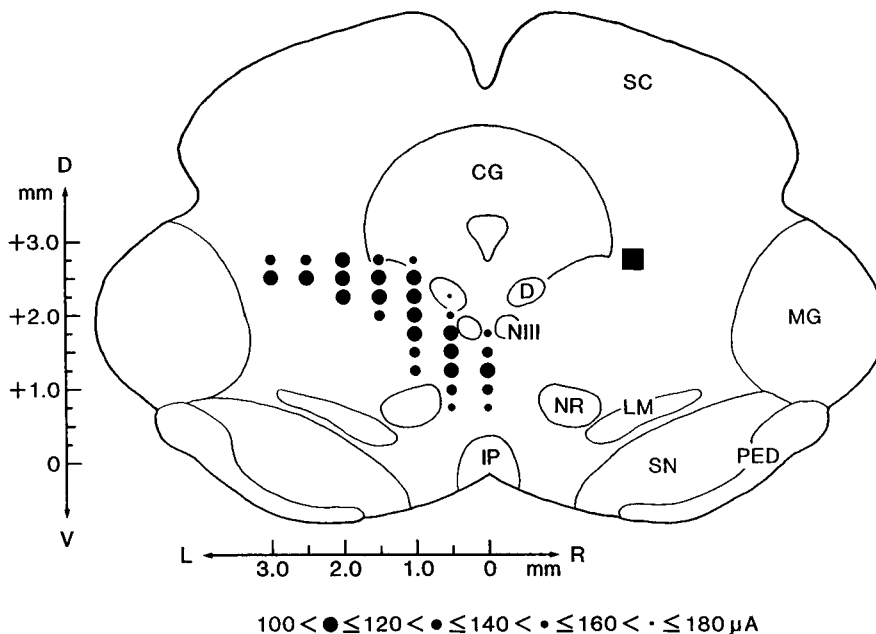
by single or short-train pulses applied to either the ipsi- or contralateral CMA.

In addition to the MRF, repetitive stimulation of the substantia nigra induced rhythmic DIG and MASS EMG activities at intensities comparable to those of MRF stimulation. However, the pattern of induced rhythmic EMG burst was similar to that induced from the CMA (Fig. 1*A*) and was clearly different from that induced from the MRF (Fig. 1, *B, C*). Furthermore, the effective sites in the MRF were separated from those in the substantia nigra by a wide ineffective zone.

Although we also tested the effects of repetitive electrical stimulation of the superior colliculus in all the 65 animals used in this study, we did not succeed in inducing RJMs in any of the animals tested.

Discussion

MRF region inducing RJMs and its descending routes responsible for RJM induction

The present study has demonstrated that repetitive electrical stimulation of the MRF can induce RJMs. Since the MRF-induced rhythmic burst activity of the anterior digastric motoneurons persists after precollicular decerebration, as well as cerebellectomy in the paralyzed preparation with the spinal cord sectioned at the C2 level, the rhythm can reasonably be assumed to be generated in the brain stem.

Chandler and Tal (1987) reported that repetitive electrical stimulation of the MRF suppressed CMA-induced RJMs in guinea pigs. On comparing the location of their MRF sites inducing this suppression (cf. figure 1 of their paper) with the RJM-inducing region in the MRF found in the present study, one notes that the latter is located directly rostral and dorsal to the former at the level of the red nucleus, and exactly corresponds to the area from which Chandler and Tal (1987) did not obtain suppression of the CMA-induced RJM.

Although the boundary between the superior colliculus and the MRF is not sharp (Edwards, 1980; Dean et al., 1982), the superior colliculus does not extend ventrally below the horizontal level of the cerebral aqueduct in rodents (Dean et al.,

1982). In the present study, no RJMs were induced by repetitive electrical stimulation of loci dorsal to the cerebral aqueduct, which supports the view that midbrain sites inducing RJMs are located in the MRF.

Similar to the MRF region inducing RJMs, a posterior mesencephalic region is known to induce controlled locomotion on a treadmill by application of low-amplitude repetitive pulses in the precollicular–postmammillary decerebrate cat (see Garcia-Rill, 1986, for review). This physiologically defined region is called the mesencephalic locomotor region (MLR) and is morphologically coextensive with the pedunculopontine nucleus in the cat and rat. Thus, the MLR is located caudal to the RJM-inducing region. The threshold intensity of repetitive pulses for locomotion induction from the MLR (0.2 msec, 10–60 μA) (Garcia-Rill et al., 1985) is far lower than that for RJM induction from the MRF (0.3 msec, not less than 100 μA). This suggests that stimulation of neurons in a wider area in the MRF is required to activate the central masticatory rhythm generator than to drive the central locomotor rhythm generator. Despite the difference in effective sites and threshold intensities for induction of respective rhythmic movements, there is a similarity in the pattern of their emergence during stimulation. Stimulation of respective MRF regions first induces an increase in tonic muscle tone in the jaw or limb muscles. After a period of time, this tonic activity gave way to rhythmic alternation of activity between the closing and opening muscles of the jaw or between the extensors and flexors of the limb. It is suggested that elevation of tonic activity level of neurons composing the central rhythm generator is necessary to drive the rhythm-generating circuit.

Tal (1987) induced RJMs by repetitive electrical stimulation of the ventral MRF close to the cerebral peduncle. He ascribed this effect to current spread to the corticobulbar projection fibers from the CMA to the masticatory rhythm generator in the bulbar reticular formation (Nozaki et al., 1986b). In the present study we could also induce RJMs by repetitive stimulation of the ventral MRF and substantia nigra which were located closely dorsal to the cerebral peduncle. However, there was a distinct

ineffective zone between the RJM-inducing region in the dorsal MRF and the ventral MRF as well as substantia nigra. In addition, the RJM-inducing effect of the dorsal MRF stimulation was not affected by the lesion of the pontine pyramidal tract that abolished the CMA-induced RJMs. Hence, the RJM induction by dorsal MRF stimulation cannot be ascribed to current spread to the corticobulbar fibers responsible for the CMA induction of RJMs.

Unilateral hemisection of the brain stem at the rostral border of the ponds abolished the RJMs induced from the MRF contralateral to the hemisection, but those induced from the ipsilateral MRF were unaffected by the hemisection. In addition, a midline section of the midbrain abolished the RJMs induced from the MRF on either side. Thus, the results indicate that a route crossing to the contralateral side at the midbrain level is responsible for the MRF induction of RJMs.

The exact route responsible for MRF induction of RJMs remains to be clarified, although it certainly descends outside the pyramidal tract to the bulbar reticular formation. It is possible that it descends within the RJM-suppression areas in the mesencephalic, pontine, and bulbar reticular formation (Chandler and Tal, 1987; Chandler and Goldberg, 1988). The RJM-suppression area reported by Chandler and Tal (1987) may include not only the neurons and/or fibers involved in RJM suppression, but also those involved in RJM induction; since the effects of activated RJM-suppressive component may overwhelm those of activated RJM-inducing component, suppression of the CMA-induced RJMs may be produced as the overall result of simultaneous stimulation of these 2 components.

Repetitive electrical stimulation of the MRF region inducing RJMs evoked a field potential that rhythmically fluctuated in the same rhythm as the induced RJM in the medial bulbar reticular formation, including the oral portion of the GCo where repetitive electrical stimulation of the CMA also evoked a rhythmic field potential in the same rhythm as the CMA-induced RJM. A central rhythm generator responsible for the CMA-induced RJM was reported to be located in the GCo (Nozaki et al., 1986a). Since a lesion of this region abolished the RJM induced from the MRF as well as CMA, the central rhythm generator responsible for CMA induction of RJMs is also indispensible for the MRF induction of RJMs. It is suggested that the MRF and CMA may share a common rhythm generator.

Patterns of RJMs induced from medial and lateral MRF

The RJM induced from the MRF was characterized by a large jaw-opening movement. This will readily be envisaged from the pattern of the MRF-induced rhythmic DIG EMG burst that is much larger in amplitude than that induced from the CMA.

A remarkable difference in the pattern between the RJM induced from the medial MRF and that from the lateral MRF was that the cycle time of the medial MRF-induced RJM was significantly longer than that induced from the lateral MRF. The reasons for this difference in the cycle time are, at present, unclear. However, since this difference depends on stimulated sites but not on the parameters of stimulation, there may be 2 independent input systems from the medial and lateral parts of the MRF to the central masticatory rhythm generator.

If we compare the patterns of rhythmic jaw-muscle EMG activities induced from the CMA and those from the lateral MRF, the former is characterized by a rhythmic alternation of

DIG and MASS EMG bursts, while in the latter the MASS EMG bursts occur both in and out of phase with DIG EMG bursts.

It was reported that jaw-closer EMG activities were accompanied by jaw-opener EMG activities during jaw closing or occlusal phase in mastication in some species (Weijs and Dantuma, 1975; Gorniak and Gans, 1980; Byrd, 1981; Oron and Crompton, 1985). On the other hand, during the MRF-induced rhythmic jaw movements the nonreciprocal burst of DIG and MASS EMG was seen during the jaw-opening phase. MASS EMG activity was also present during the burst phase of DIG EMG activity induced by the CMA stimulation in the guinea pig with local tetanus in the MASS (Enomoto et al., 1987). This nonreciprocal MASS EMG activity was demonstrated to be the result of stretch reflex of the MASS evoked by the jaw opening, due to block of all the inhibitory inputs to the masseter motoneurons. It could be assumed that rhythmic jaw-muscle EMG pattern induced from the lateral MRF consists of 2 components: the centrally induced reciprocal rhythmic activity in DIG and MASS EMG, and the peripherally induced MASS EMG burst in phase with the DIG EMG burst during the jaw-opening phase of an excessively large jaw displacement. Activation of gamma motoneurons of the MASS by stimulation of the MRF may also be involved in the occurrence of this stretch reflex (Shimazu et al., 1962).

Roles of mesencephalic reticular neurons in RJM induction by electrical stimulation of the MRF

Glutamate induced RJMs when it was injected into the lateral portion of the MRF in 5 of 8 trials; it was ineffective when injected into the medial portion of the MRF in any of 3 trials. Because of the small number of trials of chemical stimulation in the present study, more evidence is needed to reach a definite conclusion of the effect of glutamate injection into the medial MRF. However, at least it can reasonably be assumed that activation of the neurons in the lateral MRF can induce RJMs and is involved in RJM induction by electrical stimulation of the lateral MRF.

To chemically induce RJMs from the lateral MRF, it was necessary to inject 4.0 μl of 50 mM glutamate (0.2 μmol). The latency of RJMs was ca. 7 min after the onset of injection over a period of 3 min. The apparently long latency of RJMs and the seemingly large volume of injection required for RJM induction may raise the possibility that the site of action of glutamate is remote from the injection site.

Sinnamon (1987) reported that injection of glutamate in a dose comparable to that used in the present study (2 M, 0.1–0.2 μl, i.e., 0.2–0.4 μmol) into the preoptic region of the rat induced stepping after a latency of 40–200 sec from the onset of injection over a period of 30 or 60 sec. This implies that locally applied glutamate requires a fairly long period to activate the central rhythm-generating circuit, although the drug rather promptly excites neurons themselves. He also reported that injection of picrotoxin into the preoptic region required even a longer latency of 7.8 min for induction of locomotion that continued without interruption for ca. 25 min. Likewise Garcia-Rill et al. (1985) reported that injection of picrotoxin (1.5 μl) into the MLR of the rat elicited locomotion at a latency of 1.5–7 min after injection at rates of 0.1–1.0 μl/min, i.e., 3–22 min from the onset of injection. The duration of picrotoxin-induced locomotion ranged from 20 to 60 min. Thus, there is a similarity in the latency and duration between the RJM induced from the lateral MRF and the locomotion induced from the MLR. By

injecting the same amount of dye into the MLR, Garcia-Rill et al. (1985) estimated that the infusion spread was limited to an area approximately 1–2 mm in size in the mediolateral direction. Accordingly, it could be assumed that 4.0 μl glutamate would spread to a region occupying 0.7–1.4 mm (i.e., 0.5–1.0 mm times the cubic root of 4.0/1.5) from the point of injection. This estimate of infusion spread roughly corresponds with the histological data that the spread of methylene blue contained in 4.0 μl of injected glutamate solution was confined in an area ca. 1 mm from the injected point. The estimate would also be consistent with the results that injection of glutamate into the medial MRF could not induce RJMs, although it was less than 2 mm apart from the lateral MRF. Thus, the long latency and duration of RJMs induced by injection of 4.0 μl glutamate would not necessarily indicate that the site of action is remote from the injection site.

Possible source of inputs to the MRF involved in RJM induction

Superior colliculus. Chandler and Goldberg (1984) reported that systemic injection of apomorphine (a dopamine agonist) into guinea pigs induced RJMs and that ablation of the superior colliculus and directly underlying region blocked the ability of apomorphine to induce RJMs. The results indicate activation of the superior collicular neurons by either disinhibition or excitation is crucial for induction of RJMs with apomorphine and suggest that the superior colliculus may be a source of activating inputs to the RJM-inducing region in the MRF. In fact, projections from the superior colliculus to wide areas in the midbrain and lower brain stem were morphologically demonstrated in rodents (Redgrave et al., 1987).

However, neither glutamate injection into nor electrical stimulation of the superior colliculus in the present study could induce RJMs. The ineffectiveness of glutamate injection can be explained by assuming that the superior collicular neurons that are involved in RJM induction are not glutamate sensitive and that their activation by apomorphine is due to disinhibition, e.g., from tonic GABAergic inhibition (Redgrave et al., 1981) exerted from the pars reticulata of the substantia nigra (Rinvik et al., 1976; Graybiel, 1978; Beckstead et al., 1979).

The neurons involved in RJM induction may be dispersed in a wide region or clustered in a circumscribed region in the superior colliculus. If the former is the case, electrical stimulation in the superior colliculus in the present study may not have activated sufficient numbers of neurons to activate the central rhythm generator. On the other hand, if the latter is the case, we may not have hit the cluster of the neurons located in the area where we did not explore. In either case, electrical stimulation of the superior colliculus would be ineffective for RJM induction.

Limbic system. Kawamura and Tsukamoto (1960) induced masticatory movements by stimulation of the cerebral cortex and the amygdala in rabbits. They found that stimulation of the amygdala could induce masticatory movements after ablation of the cerebral cortex, and stimulation of the cerebral cortex could induce masticatory movements after making a lesion in the amygdala. They proposed that the routes responsible for induction of masticatory movements from the cerebral cortex and amygdala were separate from each other.

Single or short-train pulses applied to the CMA evoked no short-latency field potentials in the MRF region inducing RJMs. This suggests that there is no massive oligosynaptic projection

to this midbrain region from the CMA. On the other hand, it was reported by means of the retrograde HRP-tracing method in rats and cats that there are massive projections to the lateral portion of the MRF from wide areas in the hypothalamus, including the lateral hypothalamic area (Morrell et al., 1981; Parent and Steriade, 1981), i.e., the feeding center (Anand and Brobeck, 1951). It can be suggested that the limbic structures, including the amygdala, activate the MRF neurons to generate the masticatory movements, indirectly via the hypothalamus and/or directly via the amygdalotegmental projection (Hopkins, 1975; Hopkins and Holstege, 1978).

We conclude that the mesencephalic reticular neurons may be involved in the expression of feeding behavior as a source of impulses activating the central masticatory rhythm generator in the lower brain stem, by integrating the activating effects from the higher brain structures, including the limbic system.

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