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Medullary reticulospinal tract mediating the generalized motor inhibition in cats: Parallel inhibitory mechanisms acting on motoneurons and on interneuronal transmission in reflex pathways

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Medullary reticulospinal tract mediating the generalized motor inhibition in cats: I. Parallel inhibitory mechanisms acting on motoneurons and on interneuronal transmission in reflex pathways.

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Abbreviations: MNs, motoneurons; INs, interneurons; MRF, medullary reticular formation; PRF, pontine reticular formation; NRGc, nucleus reticularis gigantocellularis; NRMc, nucleus reticularis magnocellularis; NRPv, nucleus reticularis parivocellularis; NRPc, nucleus reticularis pontis caudalis; NRPm, nucleus reticularis paramedianus; MLF, medial longitudinal fasciculus; PPN, pedunculopontine tegmental nucleus; IO, inferior olive; EMG, electromyogram; PSPs, postsynaptic potentials; IPSPs, inhibitory postsynaptic potentials; EPSPs, excitatory postsynaptic potentials; FRA, flexor reflex afferent; VR, ventral root; REM, rapid eye movement; Q, quadriceps; Sart, sartorius; TFL, tensor fasciae latae; Saph, saphenous; PBSt, posterior biceps-semitendinosus; ABSm: anterior biceps-semimembranosus; LG-S, lateral gastrocnemius-soleus; MG, medial gastrocnemius; Pl, plantaris; FDL, flexor digitorum and hallucis longus; Tib, tibial; Sur, sural; TA, tibialis anterior; EDL, extensor digitorum longus; SP, superficial peroneal; DP, deep peroneal; CDP, cord dorsum potentials; PAD, primary afferent depolarization; DRP, dorsal root potentials; DLF, dorsolateral funiculus; LF, lateral funiculus; VLF, ventrolateral funiculus.

Running Head: Reticulospinal control of reflex pathways to MNs.

Abstract

The present study was designed to elucidate the spinal interneuronal mechanisms of motor inhibition evoked by stimulating the medullary reticular formation. Two questions were addressed. First, whether there is a parallel motor inhibition to motoneurons and to interneurons in reflex pathways. Second, whether the inhibition is mediated by interneurons interposed in known reflex pathways. We recorded the intracellular activity of hindlimb motoneurons in decerebrate cats and examined the effects of medullary stimulation on these neurons and on interneuronal transmission in reflex pathways to them. Stimuli (3 pulses at 10 - 60 μA and 1 - 10 ms intervals) delivered to the nucleus reticularis gigantocellularis evoked inhibitory postsynaptic potentials in α-motoneurons (n=147) and γ-motoneurons (n=5) with both early and late latencies. The early inhibitory postsynaptic potentials were observed in 66.4% of the motoneurons, and had a latency of 4.0 - 5.5 ms with a segmental delay of more than 1.4 ms. The late inhibitory postsynaptic potentials were observed in 98.0% of the motoneurons and had a latency of 30 - 35 ms, with a peak latency of 50 - 60 ms. Both types of inhibitory postsynaptic potentials were evoked through fibers descending in the ventrolateral quadrant. The inhibitory postsynaptic potentials were not influenced by recurrent inhibitory pathways, but both types were greatly attenuated by volleys in flexor reflex afferents. Conditioning medullary stimulation, which was subthreshold to evoke inhibitory postsynaptic potentials in the motoneurons, neither evoked primary afferent depolarization of dorsal roots nor reduced the input resistance of the motoneurons. However, the conditioning stimulation often facilitated non-reciprocal group I inhibitory pathways (Ib inhibitory pathways) to the motoneurons in early (< 20 ms) and late (30 - 80 ms) periods. In contrast, it attenuated test postsynaptic potentials evoked through reciprocal Ia inhibitory pathways, and excitatory and inhibitory pathways from flexor reflex afferent and recurrent inhibitory pathways. The inhibitory effects were observed in both early and late periods.

The present results provide new information about a parallel inhibitory process from the medullary reticular formation that produces a generalized motor inhibition by acting on α-

and γ-motoneurons, and on interneurons in reflex pathways. Interneurons receiving inhibition from flexor reflex afferents and a group of Ib interneurons may mediate the inhibitory effects upon motoneurons.

Key words: spinal interneurons
α-γ linkage
decerebrate preparation
REM sleep
locomotion

The medullary reticular formation (MRF) is implicated in a variety of functions, such as the control of arousal level, sleep-awake cycles, locomotion and postural muscle tone.[9, 29, 50, 56, 60] While Magoun and Rhines[44] first demonstrated that stimulating the MRF exerted a general influence on motor activity in decerebrate cats, it is generally agreed that a stimulation of the MRF evokes a mixture of excitatory and inhibitory effects on muscle tone. For example, in decerebrate cats [59] as well as in unanesthetized awake cats,[15] general inhibitory effects were exerted only when strong stimuli were applied, and weaker stimuli frequently evoked a mixture of facilitatory and inhibitory responses. Moreover, brief trains of stimuli to the MRF during locomotion generally resulted in facilitation and suppression of responses in a phase dependent manner.[12, 14] In contrast, Chase et al. [10] have shown that the reticular stimulus effects on spinal motoneurons (MNs) are state-dependent, i.e., stimulating the MRF of chronic cats evoked a mixture of excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs) during awaking. But IPSPs predominated during a generalized motor inhibition of rapid eye movement (REM) sleep. Subsequent studies have suggested that such state-dependent reticulospinal effects are regulated by cholinergic-monoaminergic interaction in the brainstem.[51, 63, 65, 66, 68] Therefore, it is now considered that the effects of medullary stimulation reflect changes in the excitability of MRF neurons, which depends on the experimental animal model, the behavioral state and the neurotransmitters acting on the MRF neurons.[9, 10, 15, 65, 66]

So far as the inhibitory aspects of MRF function are concerned, MRF-induced generalized motor inhibition was associated with postsynaptic inhibition of MNs[37, 41, 42, 64, 66, 68] and presynaptic inhibition of primary afferents.[8] These inhibitory effects were mediated by inhibitory interneurons.[8, 37, 64, 66] Spike-triggered averaging studies demonstrated that a particular group of reticulospinal neurons in the nucleus reticularis gigantocellularis (NRGc), which descended in the ventrolateral funiculus, induced IPSPs in hindlimb MNs located in multiple levels of the lumbosacral segments.[64, 66] Analyses of the triggered IPSPs led to the conclusion that the inhibition is mediated by interneurons located in each level of the

lumbosacral segments. Because most descending systems and peripheral afferent systems project, to a larger extent, onto spinal interneurons rather than to MNs,[2, 35, 55] we propose that reticulospinal effects are not only modified by the activity of the MRF neurons, but can largely depend on the excitability of spinal interneurons. Consequently, reticulospinal function should also be considered from the view of reticulospinal organization of interneurons. Based on the above consideration, we decided to investigate how the medullary reticulospinal system organizes interneuronal-motoneuronal systems so that a better understanding of the inhibitory function of the MRF would be gained. Indeed, as pointed out by Jankowska,[35] there is little evidence how the reticulospinal inhibitory system, through the ventral quadrant, controls interneuronal transmission in reflex pathways to MNs. In particular, the following two major questions remain. The first involves which class of interneurons mediates the reticulospinal inhibition of MNs. The second is whether the MRF-induced motor inhibition can be attributed to the inhibitory effects upon only MNs or to a parallel inhibitory action to MNs and to interneurons in reflex pathways.

This study was designed to yield data to answer these two questions. We first determined the optimal stimulus sites in the MRF for evoking an inhibition, the time course of the postsynaptic inhibitory effects upon α- and γ-MNs of hindlimb muscles, and the descending tracts conveying the inhibitory effects. Next, we examined the reticulospinal effects upon interneuronal transmission in reflex pathways to MNs. This included examination of interneurons mediating reciprocal Ia inhibition, non-reciprocal group I inhibition (Ib inhibition), and excitation and inhibition from flexor reflex afferents (FRAs), and recurrent inhibition. By varying the intervals between the conditioning stimuli and the test stimuli, it was even possible to study the time course of synaptic activity brought about by the conditioning stimuli on the interneurons. These analyses made it possible not only to determine the interneurons mediating the reticulospinal inhibition, but also to elucidate the reticulospinal control of interneuronal transmission in reflex pathways to MNs. The preliminary results of the present study have been published as an abstract.[61]

EXPERIMENTAL PROCEDURES

Surgical procedures

This study is based on data from 32 adult cats of either sex weighing 2.2 - 3.8 kg. The cats were anesthetized with halothane/nitrous-oxide gas and the L4 - S2 spinal segments were exposed with a laminectomy. The animals were surgically decerebrated at the precollicular-postmammillary level, and the anesthesia was discontinued. The trachea was intubated, and cannulae were placed in the femoral artery to monitor the blood pressure, and in the cephalic vein for administration of pancuronium bromide (Myoblock, Organon: 0.2-0.4 mg) and epinephrine (Bosmin, Daiichi Co.). The head and the vertebrae of the thoracic and lumbar segments were fixed in a stereotaxic apparatus, and a rigid spinal frame securely held the animals by pins in the iliac crests, clamps on the dorsal processes of T1-3 and L3, and a clamp on the vertebral body of L7. Retraction of the skin permitted the formation of walls for a pool of oil, which covered the exposed lumbosacral cord.

After the surgery, the cat was allowed to assume the reflex standing posture due to decerebrate rigidity. Repetitive stimuli were then delivered to the MRF so that the optimal stimulus site for producing a collapse of decerebrate rigidity[37, 41, 44] could be determined (Fig. 1). After that, the cats were again anesthetized. The L6, L7 and S1 ventral roots were cut, and their central ends were mounted on bipolar electrodes for either recording or stimulation. The following nerves of the left hindlimb were dissected and mounted on bipolar stimulating electrodes; quadriceps (Q); sartorius (Sart); tensor fasciae latae (TFL); saphenous (Saph); posterior biceps-semitendinosus (PBSt); anterior biceps-semimembranosus (ABSm); lateral gastrocnemius-soleus (LG-S); medial gastrocnemius (MG); plantaris (Pl); flexor digitorum and hallucis longus (FDL); tibial (Tib), after giving branches to popliteus, tibialis posterior, flexor digitorum and hallucis longus muscles; sural (Sur); tibialis anterior (TA); extensor digitorum longus (EDL); superficial peroneal (SP); and deep peroneal (DP). In 5 cats the right Sur and SP nerves were also mounted on bipolar electrodes. In 21 cats the bilateral dorsal quadrants were transected at the lower thoracic (Th 10 - 12) level. The spinal funiculi were

further transected in 8 cats at the lower thoracic and upper lumbar levels (Th 10 - L 2). All of the transections were made with a sharp knife under halothane/nitrous-oxide gas anesthesia.

Throughout an experiment the temperature of rectum and the oil pool were monitored, and maintained at 36 - 37° C by using radiant heat lamps. The mean blood pressure was maintained at more than 100 mm Hg by an intravenous infusion of Bosmin (< 0.01 mg) when necessary. The end tidal CO_2 was maintained between 4 - 6%. The cats were immobilized by infusion of pancuronium bromide and were artificially respired during intracellular recording.

Stimulation and recording

This study had two parts. The goal of the first part was to verify the medullary inhibitory effects upon MNs. The second part was to elucidate the medullary effects upon interneuronal transmission in reflex pathways. Repetitive stimuli (50 Hz, 10 - 60 μA, and duration 0.2 ms) were delivered for 2 – 10 seconds though a glass micropipette with a carbon fiber and Woods metal tip[64, 66] stereotaxically inserted into the MRF (P = 6.0 to 12.0, LR = 0 to 4.0, H = -4.0 to 12.0). To examine the effects on hindlimb muscle tone, the MRF was then stimulated at 0.5 - 1.0 mm intervals in the dorsoventral, mediolateral and rostrocaudal directions. Electromyograms (EMGs) were recorded from the bilateral soleus muscles with thin, bipolar, stainless steel wires (Fig. 1). During intracellular recording of hindlimb MNs, the stimulus trains (3 pulses at 10 - 60 μA, 0.2 ms duration and 1 – 10 ms intervals) were delivered to the MRF with a frequency of 1 Hz. The effects on MNs were studied by moving the electrode by 0.5 - 1.0 mm intervals in the dorsoventral, mediolateral and rostrocaudal directions. To determine the location of descending MRF projections to the spinal cord, spinal cord transections were made in 8 cats, as described previously.

In the second part of the investigation, the effects of stimulation of the MRF on interneuronal transmission in reflex pathways to MN were examined in 21 cats. In these cats, the stimulating electrodes were fixed at the optimal sites for producing inhibition of MNs.

The bilateral dorsal quadrants of each cat were transected at the lower thoracic level. So that the effects of stimulation of the MRF on interneuronal transmission could be determined a stimulus current which was subthreshold for evoking PSPs in MNs was used. The stimulus did not produce a primary afferent depolarization (PAD) in the dorsal root or change the membrane conductance of MNs. A signal processor (Model 7T07A, Sanei Co.) was used to produce the average of a test postsynaptic potential (PSP) from (usually) 10 sweeps (Figs. 4, 6, and 8). The segmental delay of each PSP was measured from the onset of the first positive peak of the volley to the onset of the PSP.

Single rectangular pulses (0.2 ms duration) were delivered at a frequency of 1 Hz to individual hindlimb nerves. Cord dorsum potentials (CDPs) were recorded at the dorsal root entry zone at L7. The latter were used for recording incoming volleys from the hindlimb nerves, monitoring the amplitude of the stimuli applied to the peripheral nerves (expressed in multiples of the threshold strength) and recording the descending volley from the MRF. The PAD induced by the MRF stimulation was evaluated in 16 cats by recording, with bipolar electrodes, the dorsal root potentials (DRPs) from rostral filaments of the L7 dorsal root.

Glass micropipettes filled with 2M K-citrate (tip diameter 1.0 –1.5 mm, impedance 3 –10 Mohm) were used for intracellular recordings from α- and γ- MNs in the L6 - S1 spinal segments. The micropipettes were connected to a high input impedance preamplifier with negative capacitance compensation (Neurodata IR 183). The reference electrode was placed in the temporal muscles. Alpha-MNs were identified by antidromic invasion and recurrent inhibition from the ventral root, and were classified according to the pattern of monosynaptic EPSPs from group Ia fibers.[16] They were analyzed only if their antidromic action potentials and membrane potentials exceeded 60 mV (mean 71 ± 5.3 mV) and - 50 mV (mean -62 ± 5.7 mV), respectively. Gamma-MNs were identified by antidromic invasion and recurrent inhibition from the ventral root, the absence of monosynaptic Ia EPSPs from hindlimb muscle nerves, and a lower conduction velocity of the motor axon.[25] The membrane potentials of MNs were monitored with a low gain DC display. Hyperpolarizing DC current was injected

through the recording micropipette using a bridge circuit. The records were displayed on a storage oscilloscope and stored for later analysis using an FM magnetic tape recorder with a band width of 0 - 5.0KHz.

Histological controls.

At the end of each experiment the stimulus sites were marked with electrolytic microlesions which were made by passing 30 μA of DC for 30 s through the stimulating electrode. The cats were then sacrificed with an overdose of Nembutal. The brainstem was removed and fixed in 10% formalin in saline. Frozen sections, 50 μm thick, were cut in the frontal or parasagittal planes and stained with cresyl violet. The locations of the microlesions in the brainstem were identified with reference to the stereotaxic atlases of Berman,[3] and Snider and Niemer.[58] All the procedures of the present experiments have been approved in the Guide for the Care and Use of Laboratory Animals (NIH Guide, revised 1978). Every attempt was made to minimize animal suffering and to reduce the number of animals used.

RESULTS

Confirmation of the medullary inhibitory effects upon hindlimb MNs.

At the beginning of this study it was necessary to confirm the medullary inhibitory effects because stimulation of the MRF usually evokes a mixture of excitation and inhibition.[15, 52, 59] We first identified "the medullary inhibitory region", the time course of the inhibitory effects, and the descending tracts mediating these effects.

Identification of the "medullary inhibitory region. Repetitive stimuli with a current of less than 30 μA were usually sufficient to abolish the bilateral hindlimb muscle tone of the decerebrate cat (Fig. 1). The bilateral soleus EMGs were typically decreased with 20 μA stimuli (Fig. 1Ab) and were immediately abolished when 30 μA stimuli (Fig. 1Ac) were delivered to the medial part of the MRF, which corresponded to the NRGc (H = -7.0).

However excitatory and inhibitory responses were often mixed. For example, NRGc stimuli at H = -6.0 initially increased the right soleus EMG before decreasing muscle tone (Fig. 1Ab, c). Stimulation of the dorsal part of the NRGc (H = -5.0) produced a tegmental reflex (flexion of the left hindlimb and extension of the right hindlimb). Stimulation of the nucleus reticularis magnocellularis (NRMc: H = -8.0 and -9.0) tonically, and/or phasically, increased bilateral hindlimb muscle tone.

The inhibitory sites in another cat (Fig. 1Ba) were also distributed in the NRGc and in the vicinity of the medial longitudinal fasciculus (MLF). Although stronger stimuli applied to either the ventral or the lateral part of the MRF sometimes reduced muscle tone, these stimuli usually increased muscle tone or evoked tegmental reflexes. In the parasagittal plane (Fig. 1Bb) effective sites were extended rostrally to the nucleus reticularis pontis caudalis (NRPc), and caudally to the nucleus reticularis paramedianus (NRPm). In 32 cats the inhibitory sites were concentrated in the NRGc (Fig. 1C).

Characteristics of PSPs in hindlimb MNs evoked by stimulating the NRGc. Intracellular recordings were made from 147 α-MNs and 5 γ-MNs. The summary, in Table 1, shows that brief trains of stimuli delivered to the "medullary inhibitory region" (NRGc) generally evoked a mixture of EPSPs and IPSPs with a variety of latencies. However, IPSPs were dominant in both extensor and flexor MNs.

Figure 2 illustrates the typical inhibitory effects from the NRGc on α-MNs. Stimulation of the NRGc (H = -7) evoked early and late latency IPSPs in a PBSt MN (Fig. 2A) with maximum amplitudes of about 3 mV and 5 mV (Fig. 2Ac) respectively. The early IPSPs had a latency of 5.2 ms and a segmental delay of 1.6 ms, and were amplified by increasing the number of stimulus pulses (Fig. 2Ad). The late IPSPs had a peak latency of 50 - 60 ms and a duration of 40 - 50 ms. In another cat, NRGc stimulation (Fig. 2B, from H = -6 to -8) also evoked early and late IPSPs in both extensor (Q and LG-S) and flexor (PBSt) MNs. But the IPSPs were smaller when stimuli were delivered to the NRMc (H = -9 and H = -10: Fig. 2A and B) and to the NRPv (not shown). On the other hand, MRF stimulation evoked

early EPSPs in a Q MN, which preceded the early IPSPs (2nd to 6th traces, Fig. 2Ba). In addition, EPSPs straddled by the early and the late IPSPs were observed in a PBSt MN (Fig. 2Ac) and in an LG-S MN (Fig. 2Bc). We classified these as "middle EPSPs", and the peak latency was approximately 30 ms. While the middle EPSPs were evoked from the NRGc, they were produced more frequently by stimulating the NRMc.

We successfully examined the effects of NRGc stimulation on 5 γ-MNs. Recordings from one of γ-MNs in response to single pulse stimulation are illustrated in Fig. 3A. The early IPSP had a segmental delay of 1.6 ms (Fig. 3Ad). The late IPSPs (Fig. 3Ac) had a peak latency of approximately 50 ms and a duration of about 50 ms. In another γ-MN stimulating the NRGc evoked late IPSPs which were reduced by an injection of hyperpolarizing current (Fig. 3Bb). The latter also enhanced early and middle EPSPs (Fig 3Bc). The amplitude of the late IPSPs was reduced by volleys in high threshold muscle afferents (Fig. 3Bd). The effect from flexor reflex afferents (FRAs) is described later. The late IPSPs were observed in all of the γ-MNs, and the time course of all of the IPSPs, both early and late, was similar in the α-MNs and the γ-MNs (Table 2).

The early and the late IPSPs were observed in 66.4% and 98.0 % of the MNs, respectively (Table 1). In α- and γ-MNs the early IPSPs had latencies of about 5 ms and a segmental delay of 1.4 –2. 0 ms, which indicated that they were disynaptically evoked from the NRGc. The late IPSPs had latencies of 30 –35 ms and peak latencies of 50 –60 ms (Table 2). Early EPSPs were observed in 69 MNs and most of them (in 59 MNs)were classified as monosynaptic EPSPs because they had a segmental delay of less than 1.0 ms. However EPSPs in the other 10 MNs were classified as disynaptic because they had a segmental delay of more than 1.5 ms. The middle EPSPs were observed in 47 MNs.

Descending tracts mediating each PSP. Spinal cord transections were made in 8 cats to determine the descending tracts mediating each PSP. Representative results are shown in Fig. 4. Before experiments, the right half of the spinal cord and the left dorsolateral funiculus (DLF) were transected at the L1 segment. Despite this transection, stimulating the left NRGc

evoked early IPSPs, middle EPSPs and late IPSPs in an LG-S MN (Fig. 4Ab). Early EPSPs, middle EPSPs and late IPSPs were also evoked in a PBSt MN (Fig. 4Ac). After transection of the dorsal part of left lateral funiculus (LF), the middle EPSPs in these MNs were abolished (Fig. 4B). A transection of the ventral part of the LF further eliminated late IPSPs in each MN, although the early IPSPs and EPSPs were preserved (Fig. 4C). The effects of 18 spinal lesions in the 7 other cats are summarized in Fig.4D. Transections of the DLF did not affect each PSP (Fig. 4Da). However, lesions in the dorsal part of the LF attenuated or abolished the middle EPSPs (Fig. 4Db). Further transections extending to the LF attenuated the late IPSPs (Fig. 4Dc), while the early PSPs were preserved. Additional transections of the ventrolateral funiculus (VLF) did not abolish the early PSPs but eliminated the late IPSPs (Fig. 4Dd). Accordingly, the middle EPSPs were mostly mediated by fibers in the LF, while the late IPSPs were mainly mediated by fibers in the VLF. The fibers in the VLF and ventral funiculus mediated early PSPs. Stimulation of the NRGc contralateral to the MNs also evoked late IPSPs. The IPSPs were not abolished by the transection of either half of the spinal cord (not illustrated), and consequently fibers mediating the late IPSPs descend on both sides.

We investigated further to determine if each type of IPSPs was mediated by the same neuronal systems. In Fig. 5, the stimulus current for evoking the early IPSPs was lower than that for evoking the late IPSPs. Twin stimulus pulses with a variety of intervals were delivered to the NRGc so that the change in size of test IPSPs evoked by a second stimulus could be examined. The test IPSPs were enlarged by the first pulses (Fig. 5A), and more than a two-fold amplification was observed when the twin pulse interval was 50 ms (Fig. 5B). Figure 5C illustrates that the test IPSPs were amplified when the twin pulse intervals were 1 - 15 ms (early period) and 30 - 70 ms (late period). The twin-pulse test was performed in 13 MNs of 6 cats, and the same results were obtained in 9 MNs of 6 cats. The most likely interpretation of this finding is that the early and the late IPSPs are mediated by common inhibitory interneurons (Fig. 5D).

Reticulospinal control of interneuronal transmission in reflex pathways to MNs.

After studying the MRF stimulus effects upon MNs the stimulating electrode was fixed at the medullary inhibitory region. Then the effects upon interneuronal transmission in reflex pathways were examined in 21 animals. The bilateral dorsal quadrants were transected at the lower thoracic level so that contamination of the middle excitatory effects could be avoided. In addition, there was a need to demonstrate that conditioning stimuli did not produce either PAD in dorsal roots or conductance changes in MNs. Usually NRGc stimulation, which was sufficient to evoke IPSPs in MNs, produced a PAD (Fig. 6Aa). But the latter was not observed when stimuli, which were subthreshold for evoking the IPSPs (Fig. 6Ab) were used. Moreover, the conditioning stimuli did not change either the amplitude or shape of the Ia EPSPs (Fig. 6Ac-e). These observations were considered as evidence that any effects of the subthreshold stimuli could be attributed to changes in the excitability of interneurons in reflex pathways.

The conditioning NRGc stimulation in part facilitated non-reciprocal group I (Ib) inhibitory pathways to MNs. However, the stimulation generally suppressed interneuronal transmission in pathways mediating reciprocal Ia inhibition, excitation and inhibition from FRA and recurrent inhibition.

"Non-reciprocal group I (Ib) inhibitory pathways". Medullary stimulus effects upon Ib inhibitory pathways were examined in 20 MNs of 12 cats. It was observed that the NRGc stimulation often facilitated these pathways. In Fig. 6B, volleys from group I muscle afferents to the LG-S MNs evoked Ib IPSPs which were preceded by Ia EPSPs (Fig. 6Bb, d). Conditioning NRGc stimuli increased the size of the Ib IPSPs without changes in the size of the Ia EPSPs (Fig. 6Bc, e). In a Q MN, stimulation of either the NRGc or a TFL Ia afferent alone did not evoke IPSPs (Fig. 6Ca, b). But a combination of both stimuli did produce small IPSPs (Fig. 6Cc). When the stimulus intensity to the TFL nerve was high enough to activate a Ib afferent, the combined stimuli apparently produced IPSPs with a segmental delay of 1.6 ms

(Fig. 6Cd, e). The time course of the facilitatory effects was also examined (Fig. 7). Conditioning stimulation did not induce PSPs but increased the size of the test Ib IPSP (Fig. 7A). The test IPSP was maximally increased when an incoming volley from FDL overlapped with a descending volley from the NRGc (indicated by a downward arrow in Fig. 7Ba). The test IPSP was also increased when both stimuli were delivered with an interval of 60 ms (Fig. 7Bb). Figure 7C shows that the facilitation was observed in both early (1 - 20 ms) and late (40 - 80 ms) periods. Facilitation of Ib inhibitory pathways was observed in 6 of 10 extensor MNs and 3 of 10 flexor MNs in 8 cats. On the other hand, suppression of these pathways, which was prominent in the late period (40 - 80 ms), was also observed in 3 MNs. No significant change was seen in another 8 MNs. These observations suggest that a particular group of Ib inhibitory interneurons mediate the early and late IPSPs in MNs (Fig. 7D).

Medullary stimulus effects upon Ib excitatory pathways to MNs were examined in 6 MNs (4 cats). Suppression, which was marked in the late period (40 - 70 ms), was observed in 3 MNs, but an obvious effect was not found in the other 3 MNs.

"Reciprocal Ia inhibitory pathways". Inhibitory effects mediated by reciprocal Ia interneurons are depressed by recurrent inhibitory pathways through Renshaw cells.[33, 34] We examined if the latter reduced the size of NRGc-induced IPSPs. As shown in the example, stimulating the L6 ventral root attenuated Ia IPSPs in a PBSt MN (Fig. 8Aa, b). The stimulation, however, did not attenuate the NRGc-induced late IPSPs (Fig. 8Ac, d). Moreover, ventral root stimuli did not affect either early IPSPs or late IPSPs in an LG-S MN (Fig. 8B). Although ventral root stimuli exclusively depressed Ia IPSPs in all the MNs examined (12 MNs of 7 cats), these stimuli failed to depress the NRGc-induced IPSPs. Consequently reciprocal Ia inhibitory interneurons would not mediate the reticulospinal inhibition of MNs.

We further examined the NRGc stimulus effects on transmission in reciprocal Ia inhibitory pathways to 23 MNs of 14 animals. Suppressive effects were observed in 11 MNs of 10 cats. In a TA MN (Fig. 8C) conditioning stimulation did not have an effect on Ia EPSPs (Fig. 8Cc, d), but it attenuated Ia IPSPs (Fig. 8Ce, f). As can be seen in Fig. 9C, suppression

of a reciprocal Ia inhibition from Q muscles to a PBSt MN was observed in both early (2 - 20 ms) and late (40 - 70 ms) periods. The time course of the suppression was similar to that of the NRGc-induced IPSPs (Fig. 9Ad). Thus, it can be explained that NRGc stimulation activated inhibitory interneurons that, in turn, inhibited the reciprocal Ia interneurons (Fig. 8D). Suppression of the reciprocal Ia inhibition was observed in 8 of 15 flexor MNs and in 3 of 8 extensor MNs. In the 12 other MNs, no obvious effects were observed.

"Recurrent inhibitory pathways". Because recurrent inhibitory pathways inhibit both MNs and reciprocal Ia interneurons,[35, 55] we invastigated in 9 animals if NRGc stimulation facilitated transmission in recurrent inhibitory pathways to MNs. However, the NRGc stimulation apparently reduced the size of the test recurrent IPSPs in MNs induced by ventral root stimuli (Fig. 10A, B). The reduction was marked when the test PSPs were observed at 40 - 80 ms (late period) after the conditioning stimuli. A suppression of this pathway was observed in 9 of 12 extensor MNs and 7 of 10 flexor MNs. No effect was observed in the 6 other MNs. It can be considered that the NRGc stimulation activates interneurons that subsequently inhibit Renshaw cells (Fig. 10C).

"FRA pathways". Conditioning NRGc stimulation did not change the input resistance of the PBSt MN (Fig. 11Ac, d) but reduced the size of test EPSPs from volleys in high threshold muscle afferents (Fig. 11Ae, f). The reduction of the test EPSPs was observed in both early (5 - 25 ms) and late (35 - 70 ms) periods and was more prominent in the latter (Fig. 11Ag). In another PBSt MN, NRGc stimulation attenuated test EPSPs evoked by volleys in cutaneous afferents (Fig. 11Ba, b) and high threshold muscle afferents (Fig. 11Bd, d). In particular, the EPSP denoted by the arrow (Fig. 11Ba) was abolished. The conditioning stimulation also reduced polysynaptic IPSPs from volleys in FRAs in an extensor MN (Fig. 11C). Thus, the NRGc via inhibitory interneurons probably inhibits both excitatory and inhibitory interneurons in FRA pathways (Fig. 11D). In 18 cats, transmission in FRA pathways was suppressed in 24 of 33 (73 %) cases. Moreover, NRGc stimuli even attenuated PSPs from contralateral FRA pathways to 7 of 9 MNs in 5 animals (not illustrated).

To further elucidate whether interneurons mediating the medullary inhibitory effects were interposed in FRA pathways to MNs, we finally examined the effects of volleys in FRAs upon NRGc-induced IPSPs in 45 MNs (43 α-MNs and 2 γ-MNs) of 19 cats. In a PBSt MN, conditioning Tib nerve stimuli preceding the test stimuli attenuated and abolished both early and late IPSPs (Fig. 12Ac, d). The conditioning stimulation applied after the test stimulation even diminished the late IPSPs (Fig. 12Ae). Volleys in high threshold afferents also eliminated NRGc-induced IPSPs in an FDL MN (Fig. 12B). These suppressive FRA effects were observed in 38 of 43 α-MNs, and were even revealed in 2 γ-MNs (one example was shown in Fig. 3Bd). These findings indicate that reticulospinal inhibition can be largely mediated by interneurons receiving inhibition from FRAs (Fig. 12D). Moreover, volleys in FRAs sometimes enhanced early (disynaptic) and middle EPSPs in addition to suppressing the IPSPs (Fig. 12C).

DISCUSSION

This study provides valuable information concerning the mechanisms underlying the motor inhibition induced by MRF stimulation. First, medullary inhibition would be associated with a parallel inhibitory action on α- and γ-MNs and on interneurons in transmission of reflex pathways to MNs (Fig. 13A). Second, the inhibition of MNs may be mediated by interneurons receiving inhibitory inputs from FRA and a group of Ib interneurons (Fig. 13B). Before considering these two issues, we will discuss the experimental approaches in this study. The organization of the medullary reticulospinal systems and interneuronal mechanisms of motor inhibition are further discussed in relation to the control of movements.

Consideration on the experimental procedures.

The "medullary inhibitory region" was located mainly in the NRGc from which electrical stimulation suppressed postural muscle tone associated with inhibition of MNs and

interneuronal transmission in reflex pathways. Spinal cord transections revealed that the inhibitory effects were mediated by fibers descending in the ventral quadrant. Based on these findings, our conclusions in addition to those of other investigators [37, 44] are that the general, or non-reciprocal, inhibition from the MRF is mediated by fibers descending in the ventral quadrant. However, we must consider the limitations of our experimental design. Specifically, the effects of electrical stimuli applied to the MRF are difficult to ascribe to one particular neuronal system because a number of descending, ascending, and local neuronal systems must be activated. Moreover, spinal cord transections can not selectively eliminate excitatory pathways and leave the inhibitory pathways.

Considerable caution was also taken to study the medullary stimulus effects upon interneuronal systems. First, there was a need to prove that the suppression of PSPs caused by conditioning stimulation was due to the inhibition of interneuronal transmission. Since the NRGc stimulation required to produce effects on reflex pathways often evoked IPSPs in MNs, [37] test PSPs could be suppressed by increasing the membrane conductance of the MNs. Additionally, a PAD following the NRGc stimulation possibly induced presynaptic inhibition of primary afferents and attenuated the test PSPs. Second, it was necessary to confirm that the effects on reflex pathways were produced by the medullary inhibitory system and not by other descending systems. Because the MRF has a heterogeneous structure with a mixture of various sized cells and fibers, reduction in stimulus current could eliminate the contribution of the small fibers and leave only the large fibers. In this study suppression of the test PSPs by the conditioning stimulation was seen in both early and late periods. This proved that the suppression was ascribed to the inhibition of interneurons that was brought about by the activation of the medullary inhibitory system.

Organization of the medullary reticulospinal systems.

Although the present results support the notion that the MRF stimulation activates different descending systems that produce a mixture of excitatory and inhibitory effects, [15, 59]

the inhibitory effects were predominant. What mechanisms are operating in the generation of the inhibitory effects? Kohyama et al.[39] recorded the activity of fast- and slow-conducting medullary reticulospinal neurons. They proposed that two distinct reticulospinal systems from the MRF mediated early and late inhibitory effects, respectively. It has been shown that a group of reticulospinal neurons in the NRGc, descending in the VLF with a conduction velocity of 80 - 100 m/s, evoked disynaptic IPSPs in extensor and flexor MNs.[64, 67] Thus an activation of the fast-conducting reticulospinal neurons in response to the NRGc stimuli, and subsequent activation of spinal inhibitory interneurons would evoke early, disynaptic IPSPs.

Because the late IPSPs were observed in most MNs, the mechanisms of generating the late IPSPs are critical in the motor inhibition. The time course of the late IPSPs is quite similar to that of the IPSPs induced by stimulating the pontine reticular formation (PRF) and the NRGc during a period of REM sleep in unrestrained cats.[10, 20] In decerebrate cats, the late IPSPs was amplified by injecting carbachol, a long-acting cholinergic agent, into the PRF[51, 65, 68] In addition, stimulating the pedunculopontine tegmental nucleus (PPN), one of cholinergic nuclei in brainstem, evoked late IPSPs in hindlimb MNs.[61] Therefore, an activation of cholinergic systems seems to be necessary for generation of the late IPSPs. Because dense connections exist between the PPN and the pontomedullary reticular formation,[40, 48] the NRGc stimulation may activate the cholinergic PPN neurons. The latter, in turn, possibly excite slow-conducting reticulospinal neurons, which eventually activate spinal interneurons and evoke late IPSPs.

In a twin-pulse test, an amplification of early test IPSPs was seen in early and late periods. The amplification could occur at either the level of spinal interneurons or the level of the reticulospinal neurons that mediated the test IPSPs. However, the latter possibility can be rejected for the following reasons. First, each of the early and late IPSPs seemed to be mediated by the separate groups of descending fibers rather than the same groups of fibers (Fig. 4). Second, the amplification of the IPSPs was not accompanied by an increase in the size of descending volley from the NRGc (an arrow in Fig.5B). These results strongly

suggest that the amplification occurred at the level of interneurons. It follows that the early and late IPSPs can be mediated by common inhibitory interneurons (Fig. 5D). Nevertheless, taking into consideration the fact that the amplification was not observed in 4 of 13 MNs, the early and late IPSPs of these MNs was possibly mediated by separate groups of interneurons. Accordingly there may exist three types of interneurons, which mediate the early IPSPs, the late IPSPs and both IPSPs.

Early and middle EPSPs often preceded the late IPSPs. The early EPSPs are almost the same as those evoked from the medial MRF [52] and the MLF. [26] Because medullary reticulospinal fibers arborize in the intermediate region and the ventromedial part of the gray matter as well as in MN pools, [43, 66] a group of reticulospinal neurons may evoke monosynaptic EPSPs and disynaptic IPSPs in MNs. Yamuy et al. [68] demonstrated that NRGc stimulation in decerebrate cats evoked excitatory ventral root potentials followed by late inhibitory potentials. The nature of the excitatory potentials resembles to that of early and middle EPSPs. While the middle EPSPs were evoked from the NRGc, they were more frequently evoked by stimulating the NRMc. Time course of the middle EPSPs was also similar to that of EPSPs evoked from the caudal raphe nucleus. [17] Neuroanatomical studies illustrated that fibers arising from the MRMc and the caudal raphe nucleus mostly descend in the LF, [42, 46] while those from the NRGc mainly descend in the VLF. [43, 46] These findings support our data indicating that the fibers mediating the middle EPSPs descend more dorsally than those mediating the early and the late IPSPs do.

<u>Parallel inhibitory mechanisms of the reticulospinal system acting on MNs and on interneurons in transmission of reflex pathways.</u>

Stimulating the NRGc evoked IPSPs in both α-MNs and γ-MNs. Although the sample size was small (n=5), the profiles of the PSPs and time course of the IPSPs in the γ-MNs were quite similar to those in α-MNs. These findings suggest that the medullary inhibitory effects can be exerted in parallel onto α-MNs and γ-MNs, and reflects an "α-γ

linkage" from the MRF. Moreover, the NRGc-induced motor inhibition is associated with suppression of interneuronal transmission in reflex pathways. In particularly it should be emphasized that the reflex suppression was seen in both the early and late periods. Because there was a good correspondence between the time course of the reflex suppression and the NRGc-induced IPSPs in MNs, the NRGc stimulation possibly evoked early and late IPSPs in interneurons in reflex pathways. It follows that a large proportion of interneurons, as well as α- and γ-MNs can be inhibited in parallel by common inhibitory interneurons (Fig.13A). The interneurons, which could be involved, include those mediating reciprocal Ia inhibition, excitation and inhibition from FRA, recurrent inhibition. These results agree with the previous findings that MRF stimuli suppressed transmission in FRA pathways[37] and reduced Renshaw cell activity.[27] However, these are different from the demonstration that the cortico-, rubro- and vestibulospinal tracts facilitate transmission of reciprocal Ia inhibition[21, 27, 30, 31], and both excitation and inhibition from FRA[5, 28].

What kinds of interneurons then, do mediate the medullary inhibitory effects upon MNs? First, the proposed interneurons would invariably receive late excitation from the NRGc. Moreover, a proportion of the proposed interneurons may also receive monosynaptic EPSPs. The second criterion would be that the interneurons are inhibited from FRA. Because volleys in FRA attenuated the NRGc-induced test IPSPs, and the attenuation was observed when FRA volleys were applied even after the NRGc stimulation, we believe that the FRA volleys inhibit interneurons mediating the medullary inhibitory effects. However, it is also possible that the FRA volleys inhibit reticulospinal neurons mediating the test IPSPs via ascending tract neurons. Thirdly, a population of the proposed interneurons may include a particular group of Ib inhibitory interneurons. It should be noted that, in 9 of 20 cases, transmission in Ib inhibitory pathways to MNs was facilitated by the NRGc stimulation in early and late periods. However, all Ib interneurons would not necessarily contribute to the motor inhibition. In contrast, there was no suggestion of involvement of reciprocal Ia interneurons and Renshaw cells in the medullary inhibitory process. Consequently, the

proposed interneurons may belong to an as yet unknown population of interneurons, which receive inhibitory inputs from FRA and partly co-extensive to that of the Ib inhibitory interneurons (Fig.13B).

One will have a question as to whether the proposed interneurons exert inhibitory effects in parallel onto MNs and interneurons in reflex pathways. It has been shown that Ib interneurons mediate postsynaptic inhibition of MNs,[5, 36, 38, 54] a variety of interneurons[9, 11, 28, 36] and spinocerebellar tract neurons.[32] They also produce PAD.[54] Thus, an activation of Ib interneurons may exert parallel inhibitory effects upon these neurons. However, Xi et al. demonstrated that Ib interneurons projecting to both MNs and dorsal spinocerebellar tract neurons did not mediate motor inhibition induced by pontine carbachol administration.[67] Further studies are necessary to establish whether interneurons satisfying the above criteria exist, and whether they exert parallel inhibitory effects on MNs and interneurons.

With respect to the control of reflex pathways, the inhibitory effects from the NRGc can be intrinsically different from those evoked through dorsal reticulospinal tract.[17] The dorsal reticulospinal tract suppressed transmission of Ib inhibitory pathways and FRA pathways.[17] The inhibition of FRA pathways was caused by the inhibition of first order interneurons.[18] In contrast, the ventral reticulospinal tract described in this study possibly inhibited FRA pathways at the level of the last order interneurons and excited a group of Ib inhibitory interneurons.

Functional significance of reticulospinal-interneuronal systems in the control of movements.

Medullary reticulospinal neurons are active not only during REM sleep[29, 56, 57] but also during locomotion.[13] Siegel et al.[56, 57] observed that a population of MRF neurons discharged at high rates during both waking and sleeping. These findings imply that the medullary inhibitory system possibly operates during both REM sleep and wakefulness. As we noted in the introduction, it is generally agreed that the medullary stimulus effects depend on a behavioral state, which is thought to be regulated by cholinergic-monoaminergic

reciprocity within the brainstem.[29, 60, 65, 66] How then, is the inhibitory system involved in motor control during these states? There is a notion that the medullary inhibitory mechanisms are responsible for motor inhibition during REM sleep.[9, 10, 63, 66 21] It has been reported that spinal reflexes and the excitability of MNs are profoundly depressed,[9, 10, 22, 23] despite continued activity in the cortico-, rubro-, and vestibulospinal neurons during REM sleep.[1, 4] We propose that the medullary inhibitory system not only exerts postsynaptic inhibition in α- and γ-MNs but also inhibits transmission of signals from supraspinal motor centers and sensory afferents to MNs at the level of interneurons, resulting in the generalized motor inhibition during REM sleep.

On the other hand, the present findings in Figs. 11 and 12 indicate the existence of a mutual inhibitory interaction between the medullary inhibitory system and the FRA system at an interneuronal level. Because locomotor signals and volleys in FRAs during locomotion possibly produce rhythmical alteration of interneurons in FRA pathways[2, 35, 43], the mutual interaction in the two systems may contribute to the control of rhythmic movements. Coordinated muscle contractions require the integration of supraspinal influences with peripheral afferent signals at the levels of both interneurons and MNs. The medullary inhibitory system possibly reduces output gains from these neurons that receive supraspinal signals and sensory afferent inputs. Thus, the changes in the activity of the inhibitory system during on-going movements may modify the level of muscle tone and the degree of rhythmicity in movements so that the animal can produce appropriate motor behavior.

In conclusion, the parallel medullary inhibitory effects to α-MNs, γ-MNs and interneurons in reflex pathways can be basic mechanisms responsible for generalized motor inhibition during REM sleep and, in addition, motor control during wakefulness.

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A a Stimulus sites

P 9.0, L 1.2

H -5.0

H -6.0

H -7.0

H -8.0

H -9.0

MLF

NRGc

NRPv

NRMc

b 20 μA, 50 Hz, 5 sec.

c 30 μA, 50 Hz, 5 sec.

l-Soleus EMG.

r-Soleus EMG.

5 sec.

B a

H

-4

-6

-8

-10

NRGc

NRPv

NRMc

P 10.0

L 2 0 2 R

b

< 30 μA

< 40 μA

< 60 μA

no inhibit.

NRPc

NRPm

NRMc

L 1.5

P 15 10 5 0

C

P 8.5

P 9.2

P 10.0

NRGc

NRMc

MLF

NRPv

IO

L 3 2 1 0

H

-3

-4

-5

-6

-7

-8

-9

-10

-11

A a Stimulus sites

L 1.2
H-5
-7
-9
-11
NRGc
NRMc
IO
P 9.5
L 2
0

b
VRs L7,S1
20 mV
5 ms

PBSt 1.3T

PBSt 1.2T

2 mV
5 ms

c PBSt MN

-5
-6
-7
-8
-9
-10
-11

40 μA
2 mV
50 ms

d
1 pulse
2 pulses
3 pulses
1 mV
10 ms

B a Stimulus sites

L 1.0
H-5
-7
-9
-11
NRGc
NRMc
IO
P 9.0
L 2
0

b Q MN

-5
-6
-7
-8
-9
-10
-11

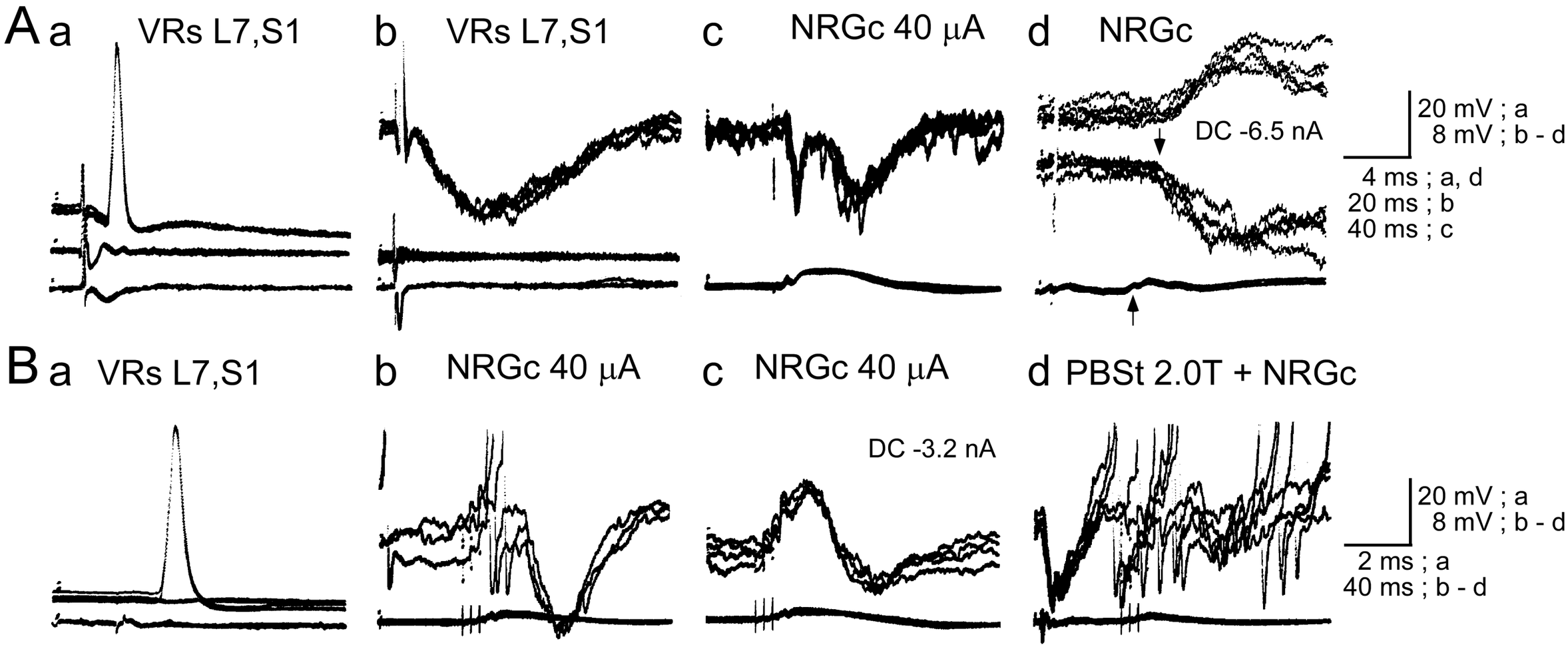
40 μA
2 mV
50 ms

c LG-S MN

40 μA
4 mV
50 ms

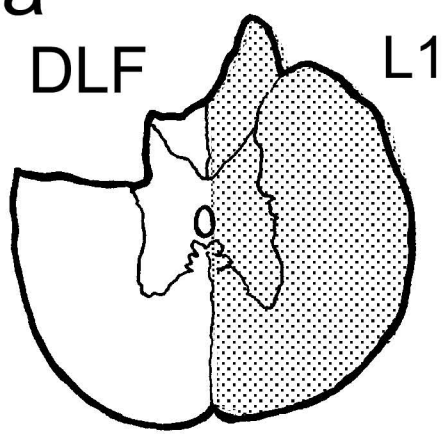
d PBSt MN

50 μA
2 mV
50 ms

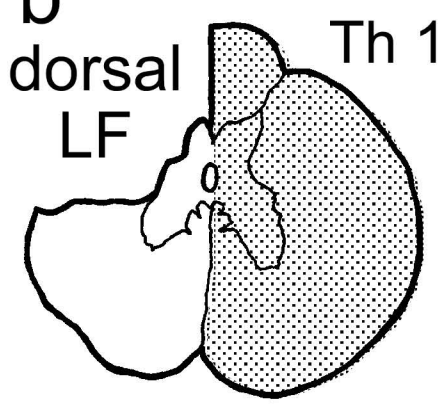

A a
VRs L7,S1
b
VRs L7,S1
c
NRGc 40 μA
d
NRGc
DC -6.5 nA
20 mV ; a
8 mV ; b - d
4 ms ; a, d
20 ms ; b
40 ms ; c
B a
VRs L7,S1
b
NRGc 40 μA
c
NRGc 40 μA
DC -3.2 nA
d
PBSt 2.0T + NRGc
20 mV ; a
8 mV ; b - d
2 ms ; a
40 ms ; b - d

A Lesions

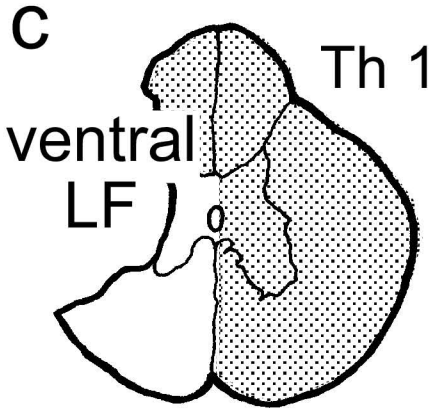
a



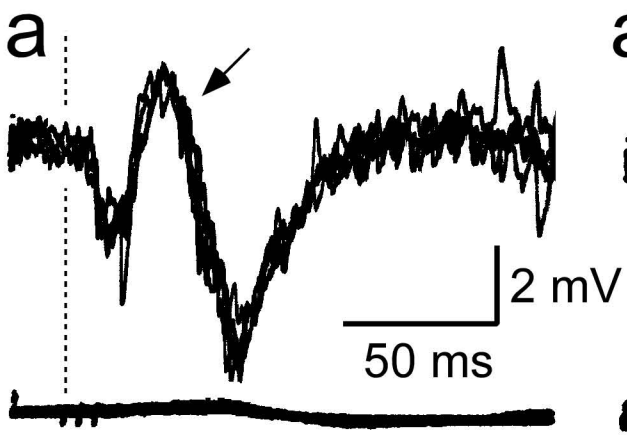
b



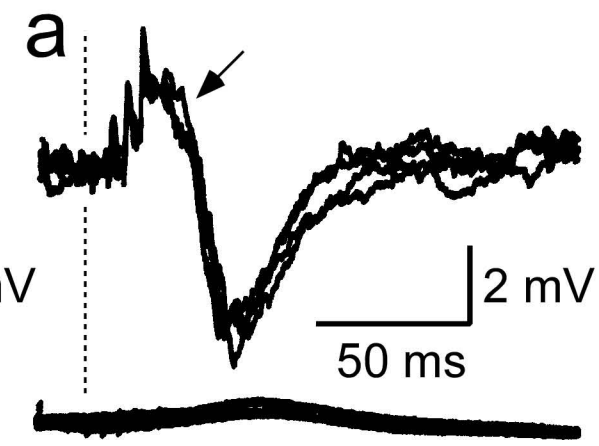
c



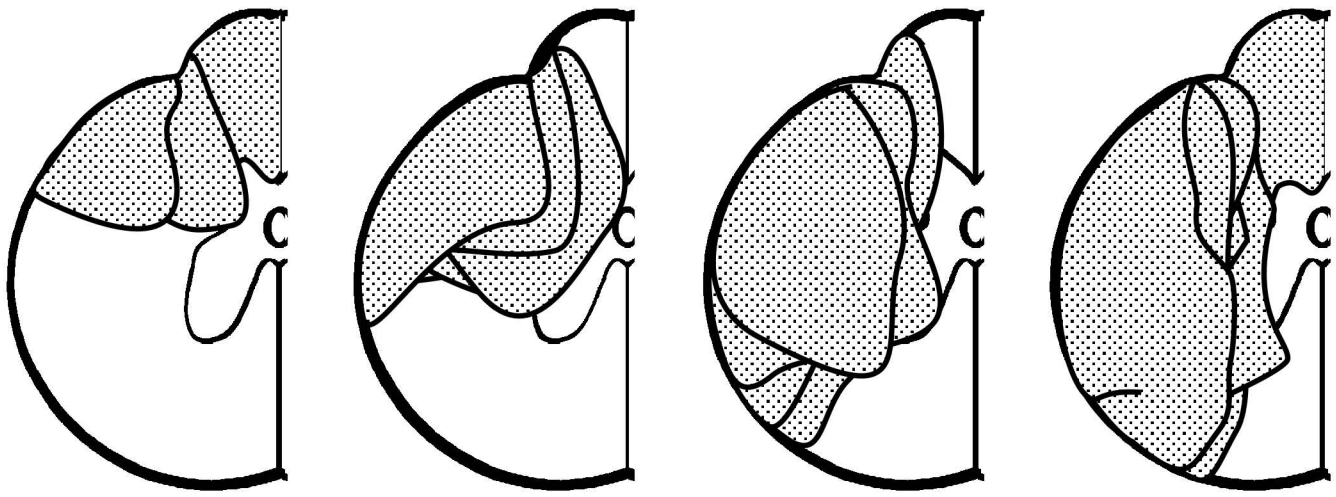
B LG-S MNs



C PBSt MNs

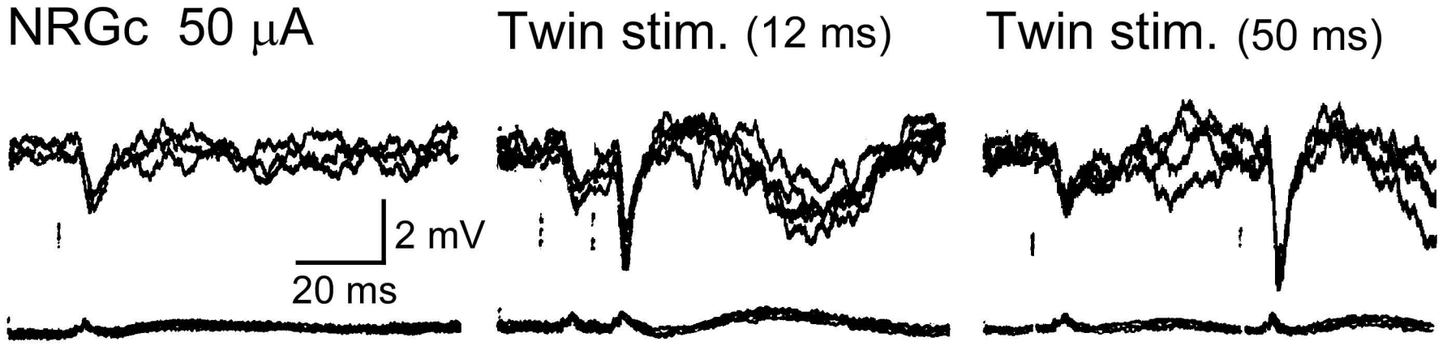


D

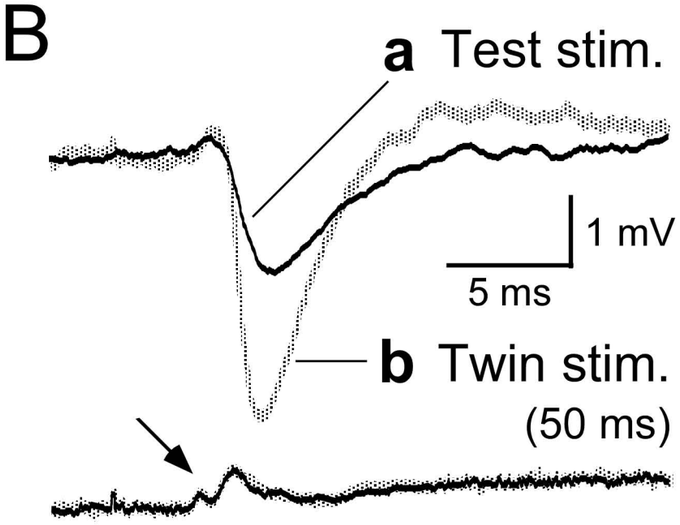


	a n=3	b n=5	c n=4	d n=6
Middle EPSPs	(+)	(±)~(−)	(−)	(−)
Late IPSPs	(+)	(+)	(±)	(−)
Early PSPs	(+)	(+)	(+)	(±)

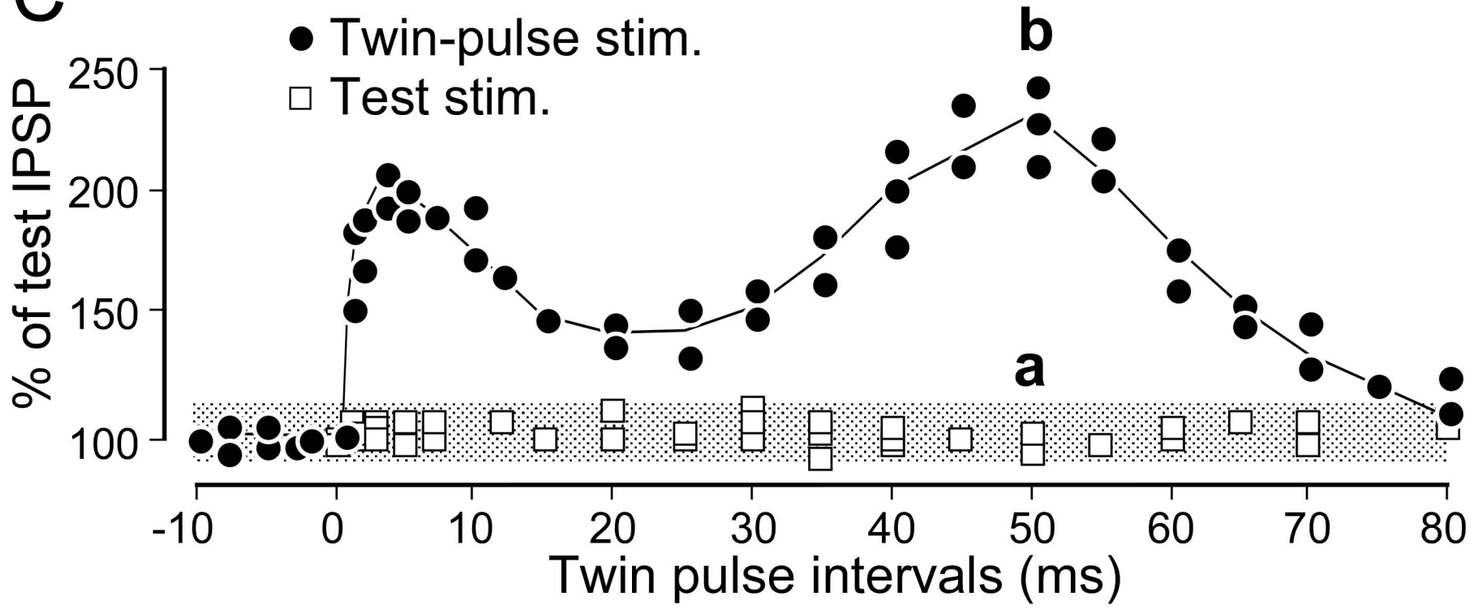
A PBSt MN


NRGc 50 μA
Twin stim. (12 ms)
Twin stim. (50 ms)
2 mV
20 ms

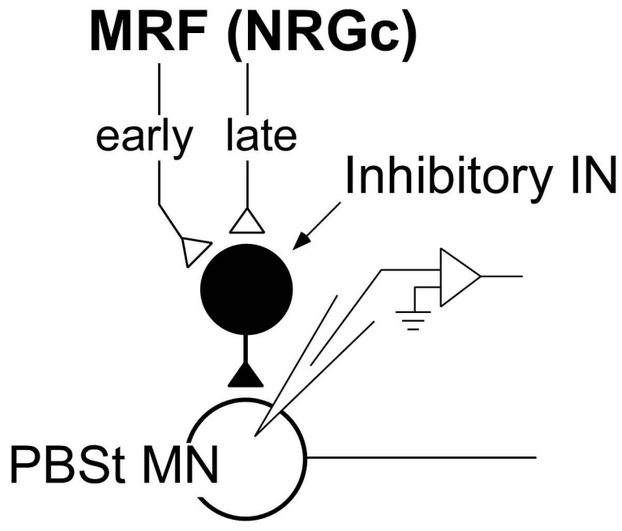
B


a Test stim.
1 mV
5 ms
b Twin stim.
(50 ms)

C


● Twin-pulse stim.
□ Test stim.
b
a
% of test IPSP
250
200
150
100
-10
0
10
20
30
40
50
60
70
80
Twin pulse intervals (ms)

D


MRF (NRGc)
early
late
Inhibitory IN
PBSt MN

A PBSt MN

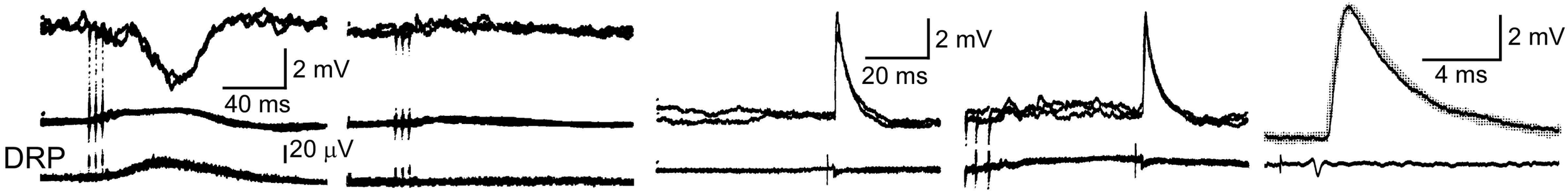
a NRGc 40 μA

b NRGc 25 μA

c PBSt 1.3T

d NRGc + PBSt

e PBSt
NRGc + PBSt



B LG-S MN

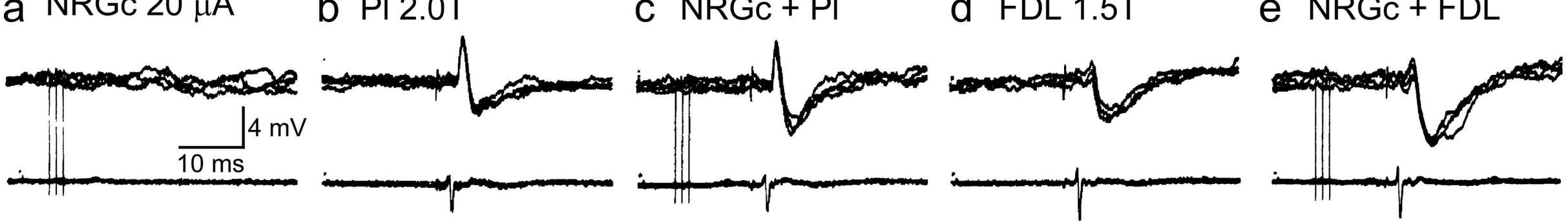
a NRGc 20 μA

b PI 2.0T

c NRGc + PI

d FDL 1.5T

e NRGc + FDL



C Q MN

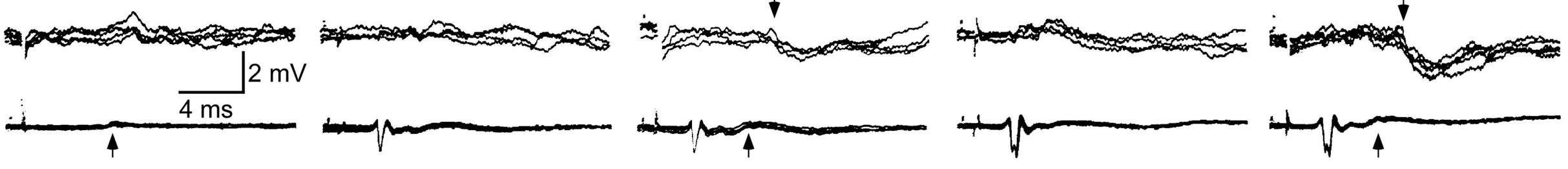
a NRGc 40 μA

b TFL 1.5T

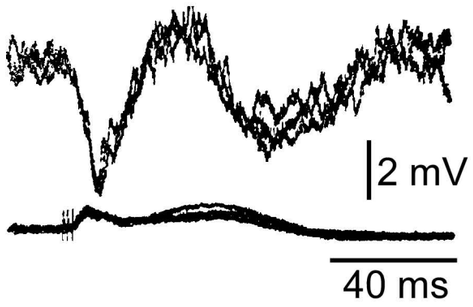
c NRGc + TFL 1.5T

d TFL 2.0T

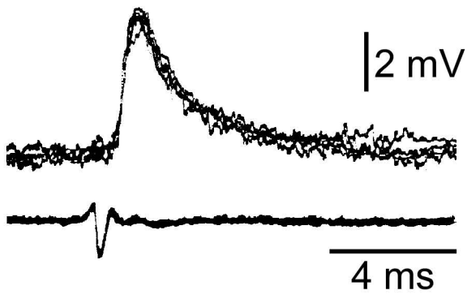
e NRGc + TFL 2.0T



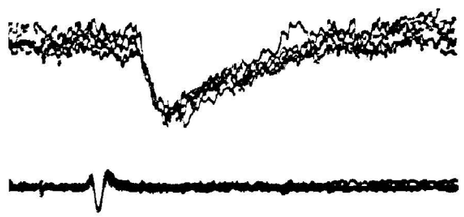
A a NRGc (3 pulses)



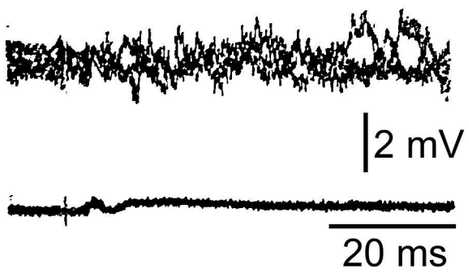
b LG-S 1.5T



c FDL 1.5T



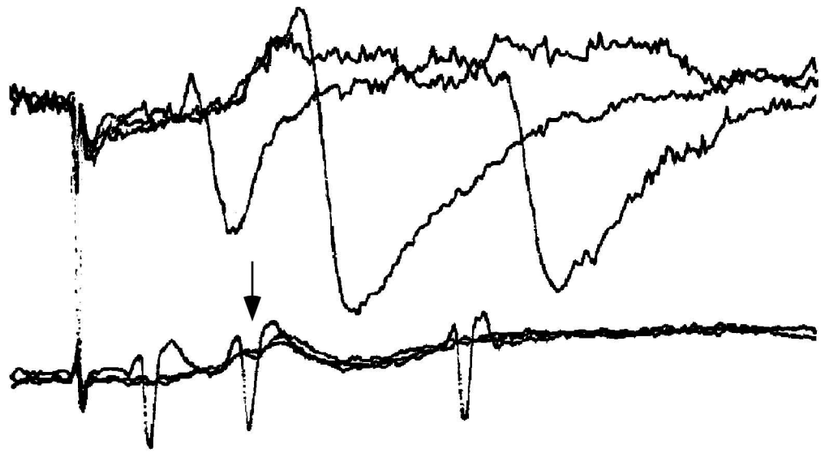
d NRGc (single)



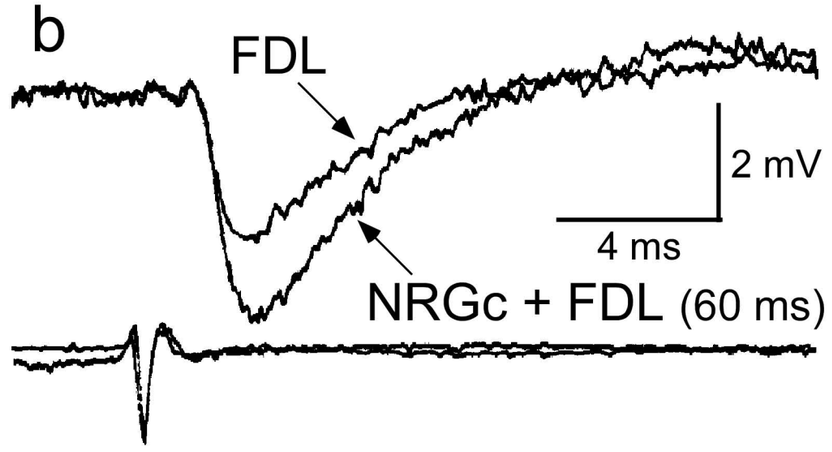
e FDL 1.5T

f NRGc + FDL

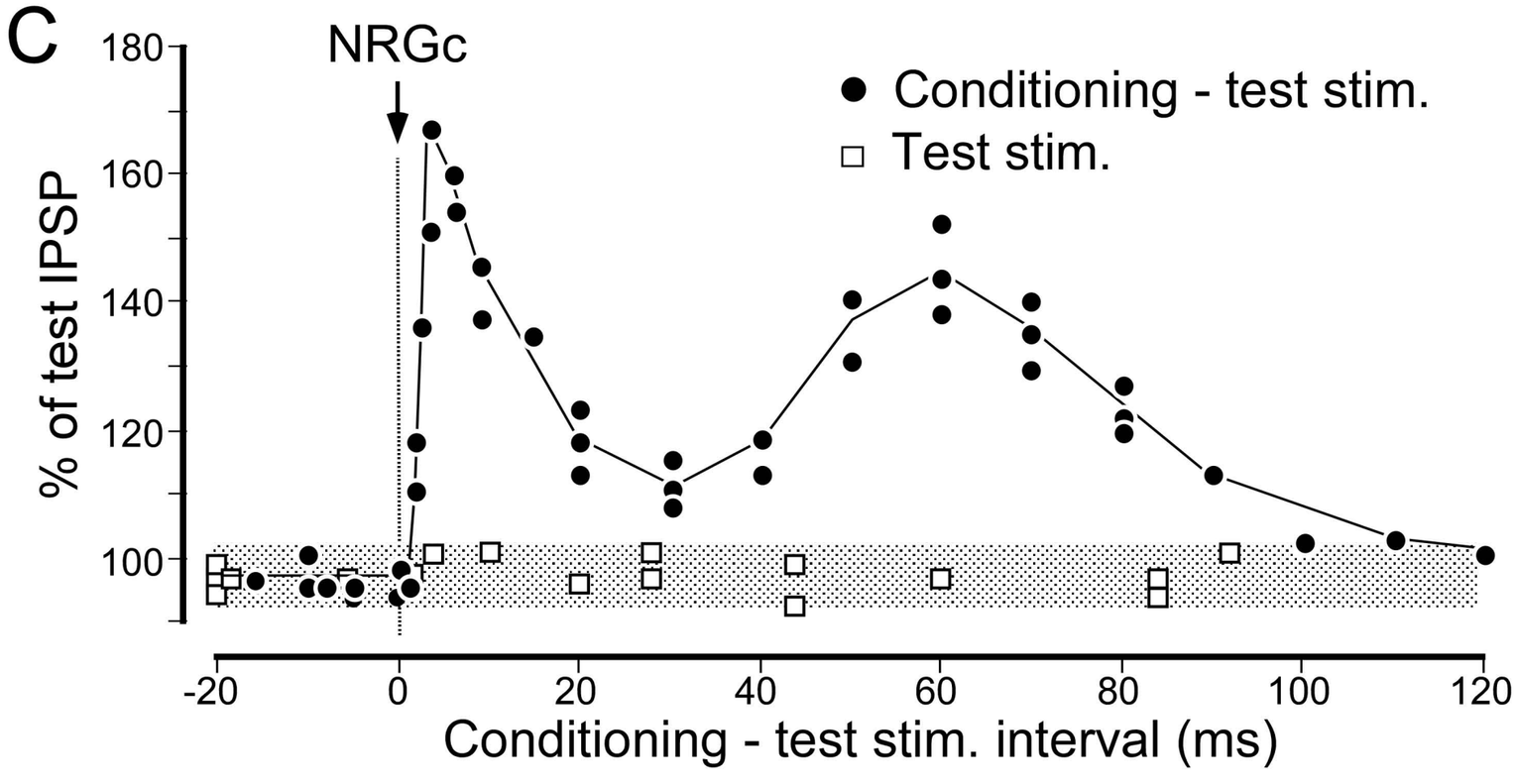
B a NRGc + FDL (0 ms, 2.5 ms, 8 ms)



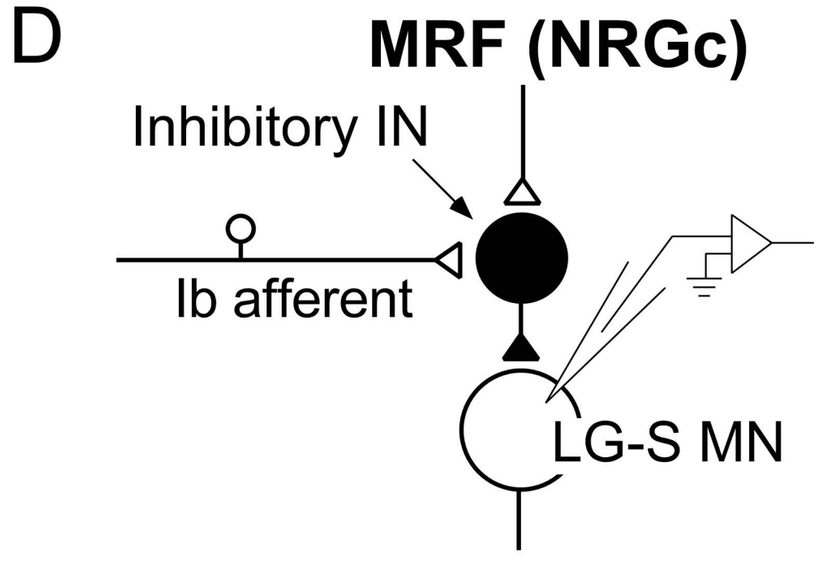
b



C



D



A PBSt MN

a Q 1.3T

b VR L6 + Q 1.3T

c NRGc 40μA

d VR + NRGc

2 mV
20 ms
2 mV
40 ms

B LG-S MN

a VRs L7,S1

b NRGc 40μA

c VRs + NRGc

d VRs + NRGc

4 mV
40 ms

C TA MN

a NRGc 25μA

b TA 1.2T

c NRGc + TA

d LG-S 1.4 T

e NRGc + LG-S

2 mV
20 ms

D

MRF (NRGc)

(C)

Inhibitory IN

R-Ia IN

ant-MN

MN

Renshaw cell

VRs stim. (A, B)

Antagonistic Ia

Homonymous Ia

A a PBSt 1.5T

b Q 1.4T

c VR L6 + Q

d NRGc 40μA

8 mV: a
4 mV: b - i
4 ms : a
10 ms : b, c
20 ms : e - i
40 ms : d

e NRGc 25μA

f Q 1.4T

g NRGc + Q (5 ms)

h NRGc + Q (25 ms)

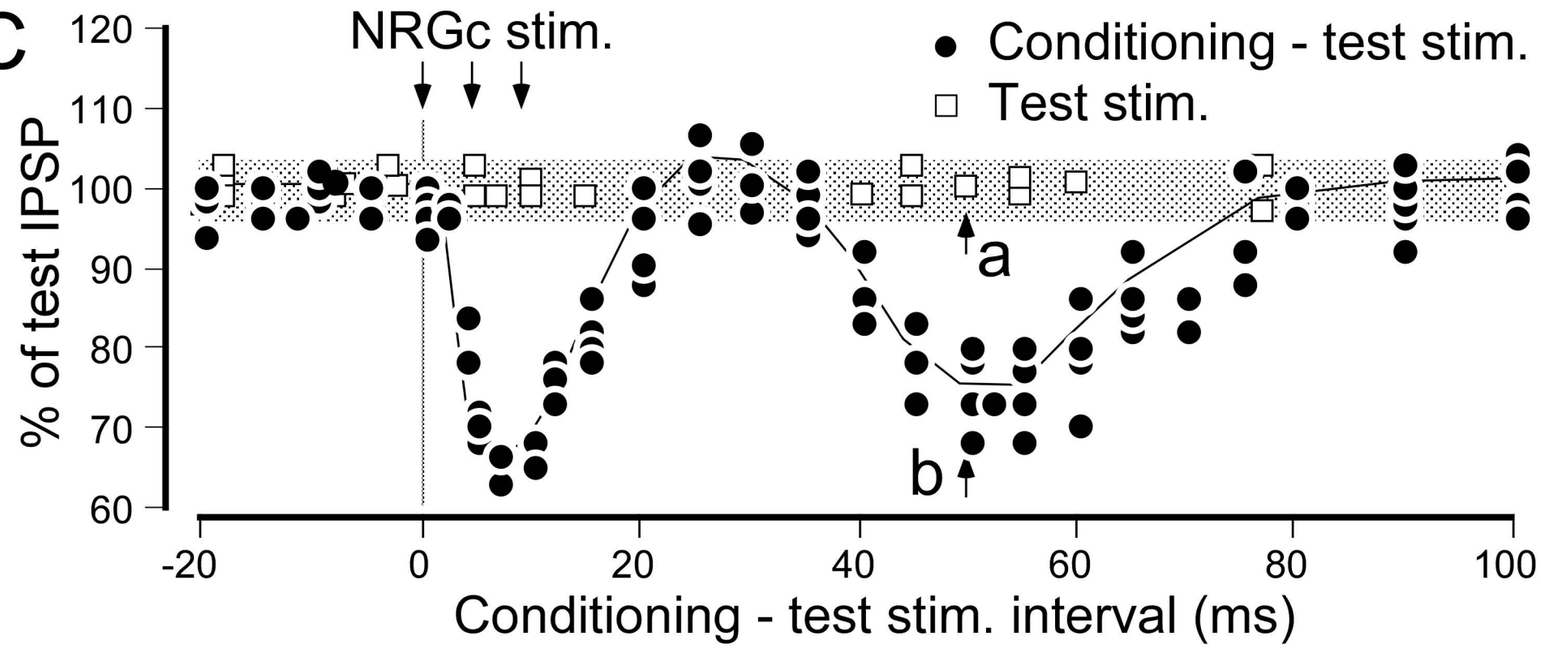
i NRGc + Q (45 ms)

B b NRGc + Q (50 ms)

2 mV
5 ms

a Q 1.4T

C



A PBSt MN

a NRGc 50 μA

b NRGc 25 μA

c VR L7

d NRGc + VR

2 mV

20 ms; b-d

40 ms; a

B LG-S MN

a NRGc 40 μA

b NRGc 15 μA

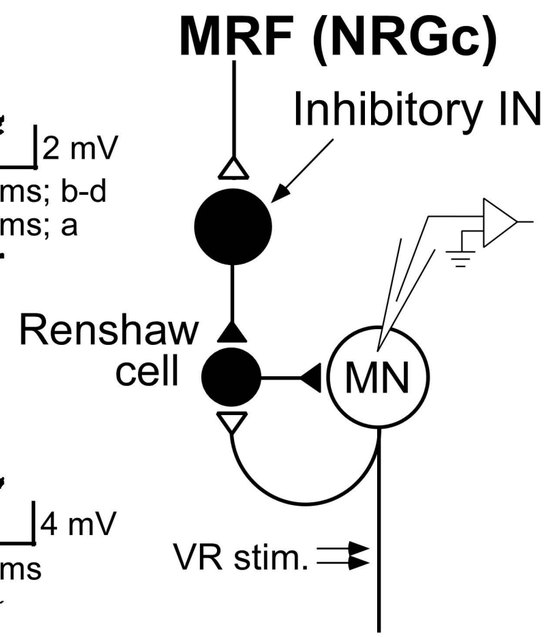
c VRs L7,S1

d NRGc + VRs

4 mV

40 ms

C



A PBSt MN

a NRGc 35 μA

b NRGc 50 μA

c Pulse 2.0 nA

d NRGc + Pulse

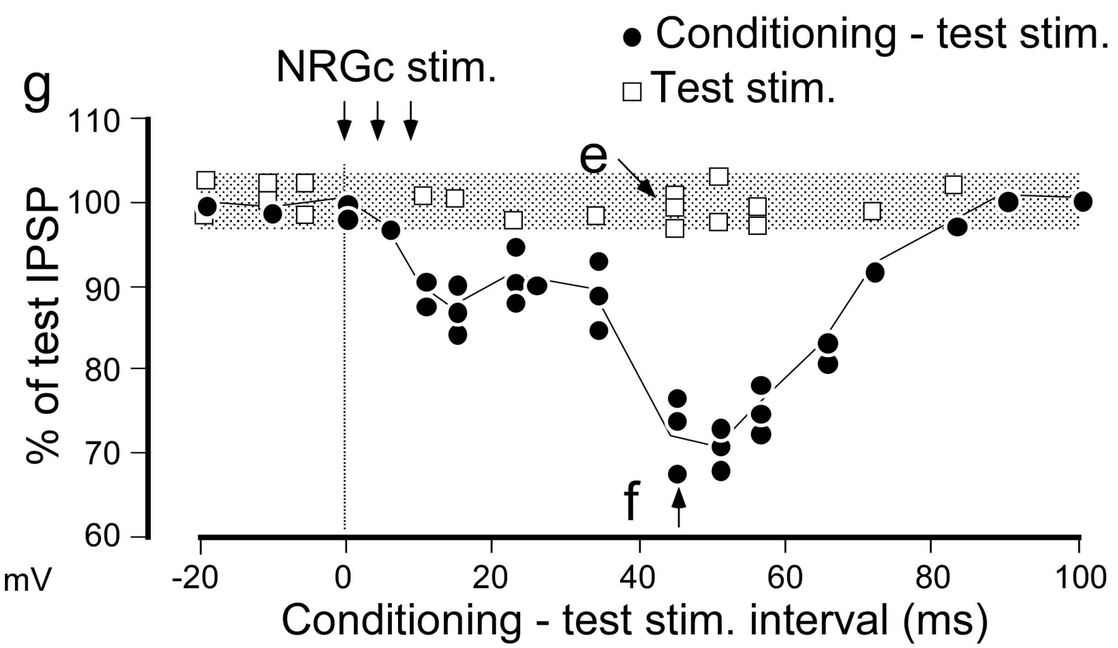
e LG-S 8.0T

f NRGc + LG-S

2 mV

40 ms

g



B PBSt MN

a SP 1.8T

b NRGc 30 μA + SP

c LG-S 10T

d NRGc + LG-S

2 mV

20 ms

C MG MN

a SP 2.5T

b NRGc 25 μA + SP

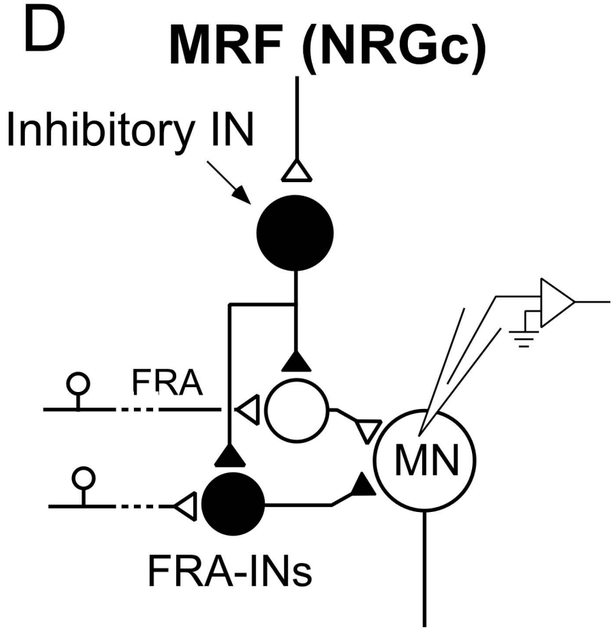
c PBSt 8.0T

d NRGc + PBSt

2 mV

20 ms

D



A PBSt MN

a Tib 1.2T

b NRGc 50 μA

c Tib + NRGc (80 ms)

d Tib + NRGc (25 ms)

e NRGc + Tib (30 ms)

2 mV

40 ms

B FDL MN

a LG-S 12.5T

b NRGc 60 μA

c LG-S + NRGc

2 mV

40 ms

C MG MN

a SP 2.0 T

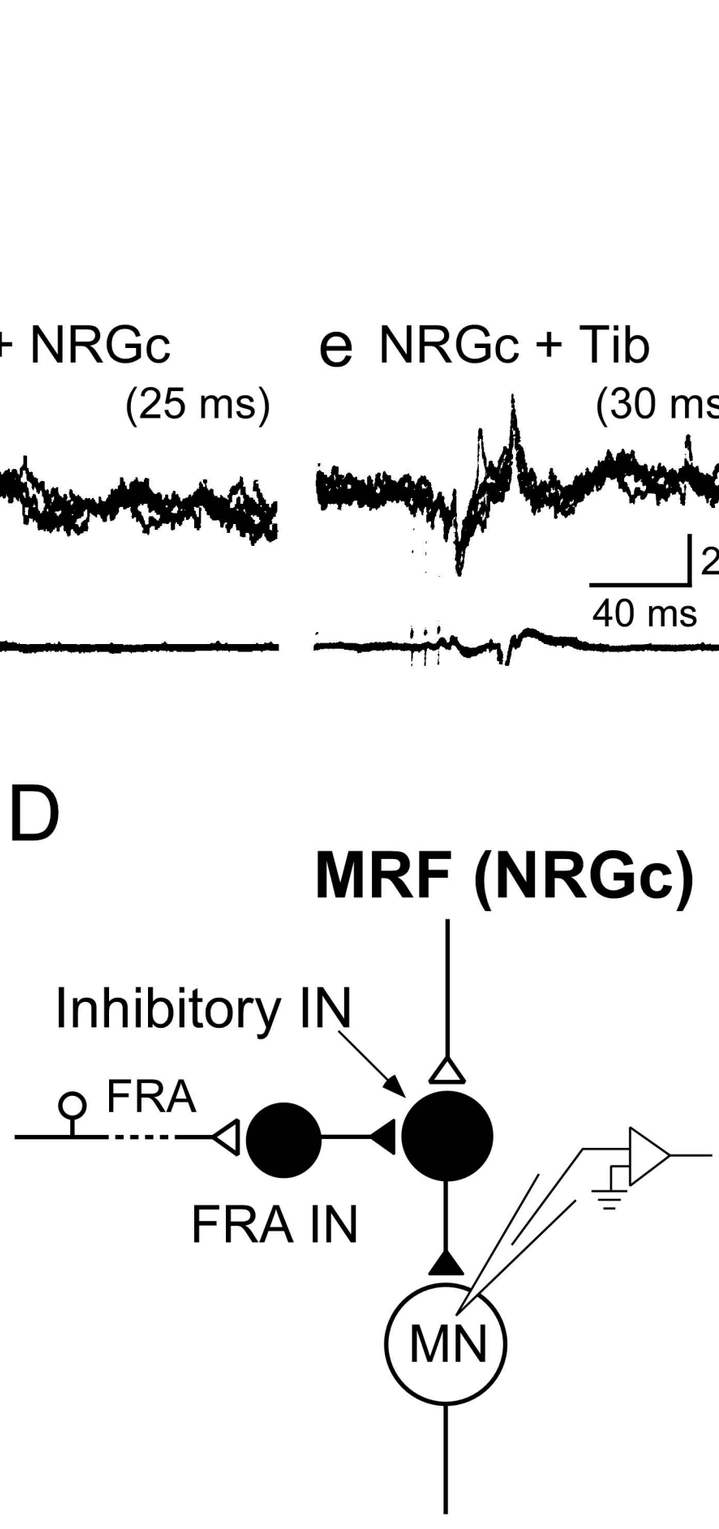
b NRGc 60 μA

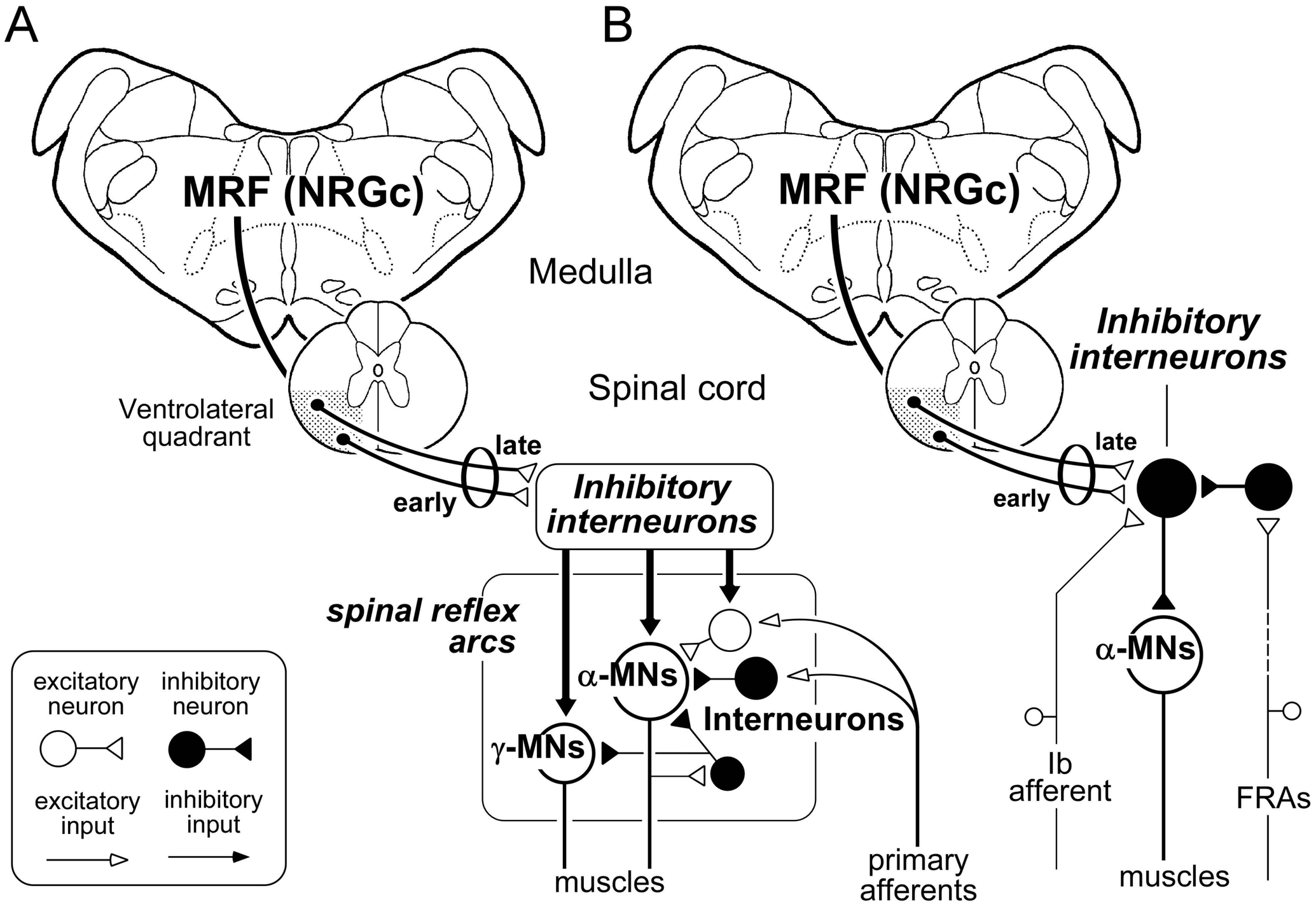
c SP + NRGc

2 mV

40 ms

D




A
B
MRF (NRGc)
MRF (NRGc)
Medulla
Spinal cord
Ventrolateral quadrant
late
early
Inhibitory interneurons
spinal reflex arcs
α-MNs
γ-MNs
Interneurons
muscles
primary afferents
Inhibitory interneurons
late
early
α-MNs
Ib afferent
FRAs
muscles
excitatory neuron
inhibitory neuron
excitatory input
inhibitory input