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Medullary reticulospinal tract mediating a generalized motor inhibition in cats: III. Functional organization of spinal interneurons in the lower lumbar segments

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**Medullary reticulospinal tract mediating a generalized motor inhibition in cats:
III. Functional organization of spinal interneurons in the lower lumbar segments**

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Running Title: Reticulospinal control of spinal interneurons

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Abbreviations

ABSm, anterior biceps-semimembranosus
CDPs, cord dorsum potentials
DP, deep peroneal
EDL, extensor digitorum longus
EPSPs, excitatory postsynaptic potentials
FDL, flexor digitorum and hallucis longus
FRA, flexor reflex afferent
IO, inferior olive
LG-S, lateral gastrocnemius-soleus
IPSPs, inhibitory postsynaptic potentials
MG, medial gastrocnemius
MRF, medullary reticular formation
NRGc, nucleus reticularis gigantocellularis
NRMc, nucleus reticularis magnocellularis
NRPv, nucleus reticularis parvocellularis
MLF, medial longitudinal fasciculus
PBSt, posterior biceps-semitendinosus
Pl, plantaris
PPN, pedunculopontine tegmental nucleus
PSPs, postsynaptic potentials
Q, quadriceps
REM, rapid eye movement
Saph, saphenous
Sart, sartorius
SP, superficial peroneal
Sur, sural
TA, tibialis anterior
TFL, tensor fasciae latae
Tib, tibial
VRs, ventral roots

Running title. Reticulospinal control of lumbar interneurons

Abstract

The previous report of intracellular recording of hindlimb motoneurons in decerebrate cats (Takakusaki et al., 2001) has suggested that the following mechanisms are involved in a generalized motor inhibition induced by stimulating the medullary reticular formation. First, the motor inhibition, which was prominent in the late latency (30–80 ms), can be ascribed to the inhibitory effects in parallel to motoneurons and to interneuronal transmission in reflex pathways. Second, both a group of interneurons receiving inhibition from flexor reflex afferents and a group of Ib interneurons mediate the late inhibitory effects upon the motoneurons. To substantiate the above mechanisms of motor inhibition we examined the medullary stimulus effects upon intracellular (n=55) and extracellular (n=136) activity of spinal interneurons recorded from the lower lumbar segments (L6–L7). Single pulses or stimulus trains (1–3 pulses, with a duration of 0.2 ms and intensity of 20–50 μ A) applied to the medullary nucleus reticularis gigantocellularis evoked a mixture of excitatory and inhibitory effects with early (< 20 ms) and late (> 30 ms) latencies. The medullary stimulation excited 55 interneurons (28.8%) with a late latency. Thirty-nine of the cells, which included ten Ib interneurons, were inhibited by volleys in flexor reflex afferents (FRAs). These cells were mainly located in lamina VII of Rexed. On the other hand, the late inhibitory effects were observed in 67 interneurons (35.1 %), which included cells mediating reciprocal Ia inhibition, non-reciprocal group I (Ib) inhibition, recurrent inhibition and flexion reflexes. Intracellular recording revealed that the late inhibitory effects were due to inhibitory postsynaptic potentials with a peak latency of about 50 ms and a duration of 50–60 ms. The inhibitory effects were attenuated by volleys in FRAs. Neither excitatory nor inhibitory effects with a late latency were observed in 69 (36.1%) cells which were located in the intermediate region and dorsal horn.

These results suggest the presence of a functional organization of the spinal cord with respect to the production of the generalized motor inhibition. Lamina VII interneurons that receive inhibition from volleys in FRAs possibly mediate the

postsynaptic inhibition from the medullary reticular formation in parallel to motoneurons and to interneurons in reflex pathways.

Keywords

Medullary reticulospinal tract,

Spinal interneurons,

Muscle tone,

REM sleep,

Locomotion

The medullary reticular formation (MRF) is involved in a variety of motor performances including eye-head coordination (Grantyn and Berthoz, 1987; Isa and Naito, 1995), locomotor movements (Drew, 1991; Drew et al., 1986; Schefchyk et al., 1984) and postural muscle tone (Mori 1987; Takakusaki et al., 1994, 2001). In the classical studies of Magoun and colleagues, stimulating the MRF exerted either a general excitatory or inhibitory influence on brainstem and spinal motoneurons (Magoun and Rhines, 1946; Rhines and Magoun, 1946). Subsequent studies however, demonstrated that MRF stimulation preferentially produced more stereotyped patterns of movements rather than generalized influences (Drew and Rossignol, 1990; Sprague and Chambers, 1954). It has also been shown that the effects of stimulating the MRF largely depend on the animal's behavioral state (Chase and Morales, 1990) and on the experimental animal preparation (decerebrate, anesthetized or chronic).

Although MRF neurons, to a certain extent, have monosynaptic connections with motoneurons of limb and axial muscles (Peterson, 1984; Peterson et al., 1978, 1979), the major effects of MRF stimulation are mediated via interneuronal relays (Jankowska et al., 1968; Drew and Rossignol, 1990; Takakusaki et al., 1989, 1994, 2001). Coordinated muscle contractions require the integration of supraspinal influences with peripheral afferent signals at the level of interneurons so that the excitability of reflex pathways and motoneurons is maintained at an appropriate level. Consequently, reticulospinal effects can be also affected by the activity of spinal interneurons. Investigations of the control of postural muscle tone in the decerebrate cat have noted that stimulating the medial MRF produced inhibitory postsynaptic potentials (IPSPs) in both forelimb and hindlimb motoneurons, with a latency of more than 30 ms. The late IPSPs possibly lead to a generalized suppression of muscle tone (Habaguchi et al., 2002; Takakusaki et al., 2001). In our previous report, which used an indirect approach and intracellular recording of hindlimb motoneurons, we have suggested that following mechanisms are involved in the MRF-induced generalized motor inhibition (Takakusaki et al., 2001). First, stimulation of

the MRF induced inhibitory effects in parallel on α - and γ - motoneurons, and on interneuronal transmission in reflex pathways to the motoneurons. Second, the inhibitory effects upon the motoneurons could be mediated by interneurons which were characterized by late excitation from the MRF and inhibition from flexor reflex afferents. The interneurons possibly included a group of Ib inhibitory interneurons.

However, to reveal the above interneuronal mechanisms of medullary-induced motor inhibition it is essential to elucidate the convergent synaptic inputs from peripheral sensory afferent systems and the descending MRF volleys on individual spinal interneurons. For this investigation we examined the effects of stimulating the MRF upon interneurons in reflex pathways by directly recording the intracellular or extracellular activity of interneurons located in the lower lumbar segments (L5–L7). In particular we attempted to elaborate three things. First, whether the MRF stimulation exerted late inhibitory effects upon the interneurons, including those mediating reciprocal Ia inhibition, non-reciprocal group I (Ib) inhibition, recurrent inhibition, and flexion reflexes. Second, whether the interneurons which were presumed to satisfy the above characteristics actually existed. Finally, the location of each interneuron within the spinal segment in relation to the medullary stimulus effects was examined. We believed this would allow us to elucidate the functional organization of spinal interneuronal systems and their relationship to the production of the generalized motor inhibition. The preliminary results of the present investigation have been published as an abstract (Takakusaki, 1998).

Experimental procedures

All the procedures of the present experiments were approved by the Animal Studies Committee of Asahikawa Medical College and are in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Guide), revised 1996. Every attempt was made to minimize animal suffering and to reduce the number of animals used. The study is based on the data from 27 adult cats (raised in an animal laboratory of Asahikawa Medical College) of either sex which weighed from 2.7 to 3.8 kg.

Surgical procedures

The animal preparation and the main experimental procedures have been described previously (Takakusaki et al., 2001). The trachea of each cat was intubated after the animal was anesthetized with halothane (Flothane, Takeda Co., Osaka, Japan) (0.5–3.0%) and nitrous oxide gas (0.5–1.0 l/min) with oxygen (3.0–5.0 l/min). A cannula was placed in the femoral artery to monitor the blood pressure and in the cephalic vein for administration of pancronium bromide (Myoblock, Sankyo Co., Tokyo, Japan) (0.1 mg/kg). The Th12–S2 spinal segments were exposed by a laminectomy. The cats were surgically decerebrated at the precollicular-postmammillary level, and the anesthesia was then discontinued. The head and the vertebrae of the thoracic and lumbar segments were fixed in a stereotaxic apparatus. The rigid spinal frame securely held the animals by pins in the iliac crests, clamps on the dorsal processes of T1–3 and clamps on the vertebral body of L3 and L7. Retraction of the skin permitted the formation of a wall for a pool of oil which covered the lumbosacral cord. The tracheal intubation was continued throughout the period of surgical procedures and the experiment, because it was useful for additional surgical operations and the control of artificial respiration during immobilization of the animal (see below).

After the surgery a cat was allowed to assume a reflex standing posture which was due to the decerebrate rigidity. Repetitive stimuli were then delivered to the MRF so

that the optimal stimulus site for evoking a collapse of the decerebrate rigidity (Jankowska et al., 1968; Llinás and Terzuolo, 1964, 1965; Habaguchi et al., 2002, Takakusaki et al., 2001) could be determined (Fig. 1B). After identification of the optimal sites for motor inhibition, the cats were again anesthetized for additional surgical operations as follows so that we could prevent unnecessary movements of the cats during the surgical operations. The experimental arrangement is illustrated in Fig. 1A. The L6, L7, and S1 (and sometimes also the L5) ventral roots (VRs) were cut and their central ends were mounted on bipolar electrodes which served as stimulation electrodes. The bipolar electrodes were 0.3 mm diameter AgCl wires separated by a distance of 2 mm. The names and abbreviations of the left hindlimb nerves which were dissected and mounted on the bipolar electrodes for stimulation are as follows: 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-off branches to the popliteus, tibialis posterior, flexor digitorum, and hallucis longus muscles; sural (Sur); tibialis anterior and extensor digitorum longus (TA-EDL); superficial peroneal (SP); and deep peroneal (DP) nerve. In 12 cats the right side of the spinal cord and dorsal funiculus of the left side of the spinal cord were completely dissected at the lower thoracic level between Th 10 and 12. A bipolar cuff electrode, of thin AgCl wires separated by 2 mm, was inserted between the spinal cord and the dura matter at the level of the L1 segment. This electrode was attached to the whole surface of the spinal cord. After these additional surgical procedures the anesthesia was discontinued.

Throughout an experiment the animal's rectal temperature and the temperature of the oil pool was monitored and maintained at 36–37 °C by using radiant heat lamps. The end tidal CO₂ was maintained between 4 and 6%. The cats were immobilized by an infusion of pancronium bromide and were artificially respired during recording from the

interneurons. The mean blood pressure was maintained at more than 100 mmHg.

Stimulation and recording

The stimulating electrode, which was a glass micropipette with a carbon fiber and a Woods metal tip (Habaguchi et al., 2002; Takakusaki et al., 2001), was stereotaxically inserted into the MRF (posterior (P) = 5.0 – 15.0, left (L) = 0.5 – 3.5, horizontal (H) = -2.0 – -10.0). To examine the MRF stimulus effects on muscle tone, repetitive stimuli (20–50 μ A, 0.2 ms duration and a frequency of 50–100 Hz) were delivered for 5 to 10 seconds. A reference electrode was placed on the temporal muscles. The MRF was then stimulated at 0.5–1.0 mm intervals in the dorsoventral, mediolateral and rostrocaudal directions so that optimal sites for suppression could be identified. The same stimulating electrode was used to examine the effects of the MRF stimulus on the lumbar interneurons. Single pulses, or stimulus trains (1–3 pulses, 2–10 ms intervals, 20–50 μ A, 0.2 ms duration), were delivered to the MRF with a frequency of 1 Hz in order to evoke postsynaptic potentials (PSPs) in interneurons, or to change the firing properties of the interneurons.

Cord dorsum potentials (CDPs) were recorded by means of a platinum ball electrode placed on the dorsal root entry zone of the rostral L7 segment against an indifferent electrode placed in the back muscles. These recordings were used for monitoring the descending volley from the MRF, and the incoming volley from the peripheral nerves. The recordings monitored the amplitude of the stimuli, expressed in multiples of the threshold strengths (xT), applied to the peripheral nerves. Individual peripheral nerves, each ventral root, and the L1 segment of the spinal cord, were stimulated every second using single rectangular pulses with a stimulus intensity of less than 1 mA and a duration of 0.2 ms.

The activity of interneurons was recorded from the left spinal segments at the levels of L6 and L7. A glass micropipette filled with 2M K-citrate solution (tip diameter 0.7–1.0 μ m, impedance 10–20 M Ω) was used for intracellular recording. The signals were

led to a pre-amplifier with a high input impedance and negative capacitance compensation (Neurodata model IR-184). The records were displayed on a storage oscilloscope and stored for later analysis on magnetic tape (FM recorder, band width 0–5.0 KHz). A low gain DC display was provided for monitoring the membrane potentials of the interneurons. A hyperpolarizing or depolarizing DC current injection was made through the recording micropipette using a bridge circuit. Glass micropipettes filled with 2M NaCl and fast green FCF were used for recording the extracellular activity which was fed to an amplifier (WPI model DAM 80) with low (300 Hz) and high (10 kHz) cut filters.

Identification of interneurons

The interneurons were differentiated from ascending tract neurons and from motoneurons by an absence of antidromic invasion induced by stimulating the L1 spinal cord and ventral roots, respectively. The strength of the incoming Ia and Ib volley was determined by the double volley technique (Bradley and Eccles, 1953). Group Ia afferents of these nerves may be expected only with stimulus intensities within the lower ranges for the first component of the incoming volleys, or up to approximately 1.2–1.3 times the threshold. Group Ib afferents were evoked by higher stimulus intensities, ranging from 1.4 to 1.5 times threshold, and appeared in parallel with the second component of the afferent volleys (Bradley and Eccles, 1953; Coppin et al., 1969; Jankowska et al., 1981b). However, it was often quite difficult to differentiate a Ia or Ib volley. High threshold muscle afferents, such as group II afferents from various muscle nerves, begin to be recruited with electrical stimuli at intensities more than 1.6 times threshold. For this reason we judged that the effects which appeared with stimuli between 2.5 and 5 times threshold could be safely attributed to afferents in the group II range (Edgley and Jankowska, 1987). In addition, the effects which appeared with a strength of 7–10 times threshold were assumed to be of group III origin (Lundberg et al., 1987a). In the present study we classified the cutaneous afferents and high threshold muscle afferents (group II

and III) as flexor reflex afferents (FRAs).

Reciprocal Ia interneurons were identified by the criteria established by Hultborn et al. (1971a, b). The criteria stated that an interneuron of this type could be identified by: (1) monosynaptic excitation by Ia afferents; (2) recurrent inhibition evoked from Renshaw cells by stimulating the ventral roots; and (3) the ability of the interneuron to follow rates of stimulation in excess of 300 Hz. Interneurons in reflex pathways from Ib afferents are co-excited by group Ia and Ib afferents and consequently some of this group are known as interneurons mediating group I non-reciprocal inhibition (Jankowska, 1992; Jankowska et al., 1981a; Czarkowska et al., 1981). In this study we defined the cells without recurrent inhibition as group I interneurons. Renshaw cells were identified by the presence of monosynaptic activation from ventral roots and by their specific, high frequency (>1000 Hz) firing pattern (Eccles et al., 1961, Renshaw, 1946). Cells which were not activated by group I afferents, but were affected by only FRA volleys, excluding Renshaw cells, were defined as FRA interneurons.

The criteria of monosynaptic excitation were as follows. When an intracellular recording procedure was used, the criteria for monosynaptic excitation were either of the following: a segmental delay of less than 1.0 ms, which was measured from the first positive peak of the descending volley from the MRF (indicated by a dashed line in Fig. 3A) to the onset of the excitatory postsynaptic potentials (EPSPs, indicated by an open arrowhead in Fig. 3A). As shown in Fig. 3A, both extracellular and intracellular recordings were obtained from several interneurons. A segmental latency, which was measured from the first positive peak of the volley to the initial spike (indicated by a filled arrowhead), was 0.2–0.3 ms longer than the segmental delay. For this reason during extracellular recording a segmental latency of less than 1.2 ms was considered to indicate monosynaptic excitation.

Histological controls

At the end of an experiment the medullary stimulus sites were marked with microlesions by passing 30 μ A of cathodal DC current through the stimulating electrodes for 30 seconds. In the lumbar segments the sites of the cell recordings were marked with fast green FCF, and when NaCl electrodes were used the electrode was left in the last recording track. An electrode for intracellular recording was also left in the last track. The cats were then sacrificed with an overdose of pentobarbital anesthesia. The brainstem and spinal cord were removed and fixed in 10 % formalin in saline. Later, frozen 50 μ m coronal sections of the brainstem and spinal cord were stained with cresyl violet. The locations of the microlesions in the brainstem were identified with reference to the stereotaxic atlases of Berman (1968) and Snider and Niemer (1961).

The position of each interneuron could be determined with respect to the last track, where the electrode remained. The depth at which each interneuron was located was calculated from the known distance from the cord dorsum and the coefficient of shrinkage of the spinal cord (5–10 %). The coefficient of shrinkage of the spinal cord, due to fixation and histological procedures, was determined from the distance between the surface of spinal cord and the location of the tip of micropipette that remained in the last track. The composite figures (Figs. 13 and 14) summarizing the location of the interneurons in the different experiments was compiled as follows. The location of an individual interneuron was marked on a drawing from the corresponding frontal section of the spinal cord. The drawings were then put together so that the contours of the grey matter and the extent of motor nuclei would fit separately for the L6 and L7 segments.

Results

Stimulation of the MRF usually evokes a mixture of excitatory and inhibitory effects on muscle activity (Sprague and Chambers, 1954; Drew and Rossignol, 1990). We therefore first identified the stimulus sites in the MRF for suppression of postural muscle tone before studying the medullary stimulus effects upon interneurons. This procedure has been described in previous reports (Habaguchi et al., 2002; Takakusaki et al. 2001). The optimal sites for suppression of the postural muscle tone of the 27 cats which were employed in this study are shown in Fig. 1B. The sites were located in the medial part of the MRF corresponding to the NRGc.

We have previously proposed (Takakusaki et al., 2001) a possible neuronal mechanism for a generalized motor inhibition, and this is shown schematically in Fig. 2. First, the medullary induced effects are exerted in parallel on α - and γ -motoneurons, and on interneuronal transmission in reflex pathways to the motoneurons (Fig. 2A). Second, the inhibitory effects upon the motoneurons could be mediated by interneurons which were characterized by late excitation from the MRF and inhibition from flexor reflex afferents. The interneurons possibly include a group of Ib inhibitory interneurons (Fig. 2B). Consequently, the medullary stimulus effects on lumbosacral interneurons were examined so that these proposals could be elucidated.

General characteristics of medullary-induced effects on interneurons

Stable intracellular and extracellular recordings were obtained from 55 cells and 136 cells, respectively. Stimulation of the NRGc was observed to induce both excitatory and inhibitory effects on interneurons (Fig. 3). The intracellular recordings revealed that stimulation of the NRGc usually induced a combination of excitatory and inhibitory postsynaptic potentials (IPSPs) in interneurons with early and late latencies (Fig. 3BC). The various combinations of early and late PSPs are summarized in Table 1. The early and late PSPs were observed in 20 and 25 interneurons, respectively. Generally early EPSPs

were followed by either late EPSPs (n=15) or late IPSPs (n=9), and early IPSPs were followed by late IPSPs (n=9). However, seven interneurons having either the early EPSPs (n=4) or the early IPSPs (n=3) were not followed by late PSPs. Early PSPs were not observed in 12 interneurons having either late EPSPs (n=5) or late IPSPs (n=7). The segmental delay of the early PSPs was measured when their onset was clearly discernible. Figure 3D displays a bimodal histogram of the segmental delay of the early PSPs. The EPSPs with a segmental delay of less than 0.8 ms (open squares, n=16) were considered to be monosynaptically evoked from the NRGc. The EPSPs with a segmental delay of more than 1.3 ms (hatched circles, n=10) were considered to be disynaptically evoked from the NRGc. The early IPSPs in 10 cells had a segmental delay of 1.4-2.0 ms which indicated that they were evoked by disynaptic connections from the NRGc. A bimodal distribution was also observed for the segmental latency of the extracellularly recorded interneurons (Fig. 3E). The cells which responded to the NRGc stimuli with a segmental latency of 0.6-1.2 ms were considered to be evoked by monosynaptic connections from the NRGc, whereas cells with a segmental latency of 1.6-2.3 ms were considered to be evoked by disynaptic connections. In addition to the early PSPs the NRGc stimulation evoked late PSPs (Fig. 3BC). The shape parameters of the late PSPs are summarized in Table 2. The late EPSPs had a peak latency of approximately 50 ms and a duration of 50-70 ms. The late IPSPs also had a peak latency of approximately 50 ms and a duration of 40-50 ms. In total, late excitatory effects were observed in 55 cells and late inhibitory effects were observed in 67 cells (Fig. 4Aa).

Reticulospinal excitation of interneurons

Representative recordings of interneurons which were excited from the MRF are shown in Figs 5, 6, and 7. The recordings displayed in Fig. 5 were from 2 interneurons in the dorsal part of lamina VII. Single pulses of stimuli to the NRGc induced monosynaptic (Fig. 5Aa) and late EPSPs (Fig. 5Ab) in the cell shown in (A). Short trains of 3 stimulus

pulses induced temporal and spatial summation of the early and late EPSPs, which resulted in burst firing of the interneuron (Fig. 5Ac). The interneuron represented in Fig. 5B also received monosynaptic and late EPSPs (Fig. 5Ba–b). Each of the cells received monosynaptic EPSPs and disynaptic IPSPs from group I muscle afferents (Fig. 5Ad–h, Bc–f). Both of the cells also received prominent inhibitory effects from the volleys in cutaneous afferents (Fig. 5Ai, Bg). Because of an absence of recurrent inhibition from the L6 and L7 VRs (Fig. 4Aj, Bh) the cells were identified as group I interneurons.

Each of the records displayed in Fig. 6 was observed from different neurons which were located in the ventral part of lamina VII. Both of the cells, like the cells illustrated in Fig. 5, received monosynaptic (Fig. 6Aa, Ba) and late excitation (Fig. 6Ab, Bb) from the NRGc. However, they lacked monosynaptic excitation from group I muscle afferents, because no excitation was observed by nerve stimuli with more than 1.5 times threshold (Fig. 6Ac–e, Bd–f). But both cells preferentially received inhibitory effects from volleys in FRAs. The cell illustrated in Fig. 6A was inhibited by volleys in group II afferents (Fig. 6Ac–e) and a cutaneous afferent (Fig. 6Af). In a different cell which is shown in Fig. 6B, trains of stimulus pulses induced a burst of firing which lasted for more than 100 ms (Fig. 6Bb). However, the discharge was removed by conditioning volleys in FRA (Fig. 6Bc). The effects of FRA inhibition on the interneurons could therefore be detected in this manner when interneurons were recorded extracellularly. These findings indicated that the interneurons received monosynaptic and late excitation from the NRGc. It was observed that one group of cells received monosynaptic excitation from group I afferents (Fig. 5C) but another group of cells did not (Fig. 6C). However both groups of cells received inhibitory effects from volleys in FRAs (Figs. 5C and 6C).

We then examined the optimal sites for evoking excitatory effects upon the interneurons (illustrated in Fig. 7). The effects of moving a stimulating electrode dorsoventrally in intervals of 1 mm on two interneurons are shown in Fig. 7A. One interneuron, illustrated in Fig. 7D, received monosynaptic excitation from a group I

muscle afferent from an LG-S muscle. Another interneuron, which is shown in Fig. 7B and C did not receive group I muscle afferents but received inhibitory input from FRAs, because the NRGc-induced discharges (Fig.7Ba–b) were abolished by volleys in FRAs (Fig.7Bc–d). Prominent early and late excitatory effects were induced in each cell when stimuli were delivered to the NRGc (H -6.0 and 7.0, the third sets of recordings in Fig. 3Ca, b and 3D). When stimuli were applied to either dorsal (H -5.0) or ventral (H -8.0 – -9.0) sites, the excitatory effects were attenuated. A mapping study was performed on another 15 cells and essentially the same findings were obtained. Generally the excitatory effects were prominent when stimuli were applied to the NRGc, but stimulating either the ventral or lateral part of the MRF did not evoke excitatory effects.

Reticulospinal inhibition on selected groups of interneurons in reflex pathways

Next we elaborated the reticulospinal effects on selected groups of interneurons, including Renshaw cells, in transmission in the reflex pathway (Fig. 4B).

Interneurons mediating reciprocal Ia inhibition: Figure 8A displays details of an interneuron which was monosynaptically excited from a Q Ia afferent and disynaptically inhibited by a PBSt Ia afferent (Fig. 8Aa, b). Because ventral root stimulation induced recurrent IPSPs (Fig.8Ac), this cell was classified as an interneuron mediating reciprocal Ia inhibition (Hultborn et al. 1971a, b). Stimulating the NRGc induced early and late IPSPs with a polarity which was reversed by injecting hyperpolarizing current (Fig. 8Ad). It is worth noting that volleys in high threshold muscle afferents from an LG-S muscle greatly reduced the amplitude of the NRGc-induced IPSPs (Fig. 8Ae, f). The possible convergent synaptic inputs to this cell are schematically illustrated in Fig. 8B. Both the early and late IPSPs could be induced by an inhibitory interneuron that receives inhibitory effects from FRAs. Figure 8C illustrates another example of a PBSt-coupled reciprocal Ia interneuron which received recurrent inhibition (Fig. 8Ca-b) and reciprocal Ia inhibition from a Q muscle (Fig. 8Cc). This cell was excited by volleys in cutaneous afferents (Fig.

8Cd). It was observed that the spontaneous discharge of this cell (Fig. 8Ce) was immediately suppressed by stimulating the NRGc (Fig. 8Cf). Among eight Ia interneurons examined the activity of 7 cells was suppressed by NRGc stimulation (Fig.4Bb). One cell did not change its firing properties in response to NRGc stimulation.

Group I interneurons: We also investigated the reticulospinal effects on 39 group I interneurons. Of the 13 cells which received late excitatory effects 10 cells also received monosynaptic excitation from the NRGc, as already shown in Fig. 5. On the other hand, NRGc stimulation inhibited the activity of 16 cells. However, 10 cells did not respond to the NRGc stimulation with a late latency (Fig. 4Bc). The cell illustrated in Fig. 9A was monosynaptically excited by group I afferents from PBSt muscles (Fig. 9Aa, b) but it lacked recurrent inhibition (Fig. 9Ac). This cell had a spontaneous discharge (Fig. 9Ae). Stimulating the NRGc induced early and late IPSPs and suppressed the spontaneous firing (Fig. 9Af). The cell illustrated in Fig. 9B was an MG-coupled group I interneuron which was recorded extracellularly. Monosynaptic excitation of this cell was not suppressed by stimulating the VRs (Fig. 9Ba-c). Spontaneous firing of this cell (Fig. 9Be) was also suppressed by NRGc stimulation for a period of approximately 120 ms (Fig. 9Bf). Both cells received excitation from cutaneous afferents (Fig.9Ad, 9Bd). Because group I interneurons receiving late inhibitory effects were located in the intermediate region (lamina IV–VI), intermediate group I interneurons could receive early and late inhibitory effects from the NRGc via inhibitory interneurons (Fig. 9C). These findings suggest a topographical organization of the reticulospinal control of group I interneurons, i.e., there is a tendency that group I interneurons in the intermediate region are inhibited by the NRGc (Fig. 9), while those located in the lamina VII are excited by the NRGc (Fig. 5).

Renshaw cells: The cell illustrated in Figure 10 was classified as a Renshaw cell by the presence of burst firing which was induced by stimulating the ventral roots (Fig. 10Aa, b). Trains of NRGc stimuli evoked both early and late IPSPs (Fig. 10Ac). The amplitude of the early IPSP increased as the number of stimulus pulses was increased (Fig. 10Ad). The

early IPSPs were considered to be induced by disynaptic linkages from the MRF because they had a segmental delay of 1.4 ms (Fig. 10Ae). Both the early and late IPSPs in this Renshaw cell could therefore be mediated by inhibitory interneurons (Fig.10B). A late IPSP was also observed in an intracellular recording from another Renshaw cell.

Extracellular recordings were obtained from two other Renshaw cells. An arrest of the spontaneous firing of these neurons by NRGc stimulation was observed for a period of approximately 100 ms from the stimulus onset. Thus NRGc stimulation induced the late inhibitory effects on all of the 4 Renshaw cells (Fig. 4Ba).

FRA interneurons: Based on the criteria described in the experimental procedure we classified 140 cells as FRA interneurons (Fig.4Bd). Of these cells 58 (41.4 %) did not respond to NRGc stimulation. These unresponsive cells were located mainly in the dorsal horn and the dorsal part of intermediate region. On the other hand, most cells recorded from the ventromedial part of the grey matter (lamina VII and VIII) received late inhibitory effects from the NRGc (n=40, 28.6%).

Fig. 11A shows an example of an FRA interneuron recorded from lamina VII. This cell was not excited by group I muscle afferents (Fig. 11Aa) but excited by both high threshold muscle afferents (Fig. 11Ab–c) and cutaneous afferents (Fig. 11Ad). Single NRGc stimulation induced early and late IPSPs with a polarity which was reversed by injecting a hyperpolarizing current (Fig. 11Ae–f). The segmental delay of the early IPSP was 1.6 ms (Fig. 11Af). Another cell which was recorded in lamina VI, illustrated in Fig. 11B, received an excitatory input from a cutaneous afferent of SP (Fig. 11Ba). The late IPSPs evoked by the NRGc (Fig. 11Bb) were greatly reduced in size (Fig. 11Bc, e) by volleys in cutaneous afferents from the SP and Saphenous nerves (Fig. 11Ba, d). This observation suggested that the medullary inhibitory effects on this cell were inhibited by volleys in cutaneous afferents. The interneuron illustrated in Fig. 11C, which was recorded at lamina VI, was excited from group II afferents (Fig. 11Ca, b) and cutaneous afferents. The amplitude of late IPSPs (Fig. 11Cc), which were induced by stimulating the NRGc,

were reduced by volleys in a group II muscle afferent (Fig. 11Cd). A total of 19 FRA interneurons were tested for the effects of FRA stimulation on the NRGc-induced IPSPs. In all of these neurons FRA volleys reduced the size of the IPSPs. These findings suggest that these FRA interneurons receive early and late inhibitory effects from the NRGc through inhibitory interneurons that are possibly inhibited by volleys in FRAs (Fig. 11D).

Stimulation of the medulla induced late excitatory effects in 42 (30.0%) FRA interneurons (Fig.4Bd). Intracellular recording of 16 interneurons revealed that these cells received either a mixture of excitatory and inhibitory effects (7 cells) or only inhibitory effects (9 cells, an example is shown in Fig. 5A) from volleys in FRAs. With extracellular recording, 26 cells in this group were excited from NRGc. These cells were judged to receive inhibitory inputs from FRAs, because the excitatory effects were diminished by conditioning volleys in FRAs, as shown in Figs. 6B and 7B.

Because interneurons receiving FRAs may be used to mediate centrally-initiated complex motor synergies (Jankowska, 1992; Lundberg et al., 1987a), it was interesting to elucidate the relationship between the effects on the interneurons from the NRGc and from FRAs. Figure 12A shows a summary of the relationship for all the interneurons that were examined intracellularly. Volleys in FRAs induced either EPSPs (n= 19) or IPSPs (n=13) or a mixture of both (n=23). It is apparent that interneurons which received excitation from the FRAs tended to be inhibited by NRGc stimulation, while those which received inhibition from the FRAs were excited by NRGc stimulation. There was therefore, an inverse relationship in the synaptic effects from the NRGc and from FRAs on the interneurons.

Figure 12B shows extracellular recordings of a relationship between the spontaneous firing rate of interneurons and the late effects from the NRGc. Generally, silent cells with a spontaneous firing frequency of less than 1 Hz received the late excitatory effects from the NRGc (n=30/35, 85.7%), while cells with a spontaneous discharge tended to receive either late inhibitory effects (n=38/42, 90.5%) or were not

affected (n=52/59, 88.1%).

Location of interneurons with respect to the responses from the NRGc

Figure 13 shows the location of all the interneurons examined in this study. The interneurons which were recorded intracellularly (large circles) and extracellularly (small circles) are plotted on coronal sections of the L6 and L7 segments. Cells with a monosynaptic excitation from the NRGc (n=61) were located in the ventromedial portion of the grey matter corresponding to lamina VII and VIII. (Fig.13A). Cells with a late excitation from the NRGc (n=55) were located in an intermediate region and the ventromedial grey matter (Fig. 13B). Interneurons which received a late inhibition (n=67) were located in the intermediate region and ventral horn (Fig.13C). Cells without late effects were distributed in the dorsal horn and intermediate region (Fig.13D). These findings suggest the presence of a topographical arrangement of the interneurons with respect to the NRGc-induced motor inhibition (Fig.14A).

In a previous study, we proposed that interneurons having the following criteria mediate the NRGc-induced generalized motor inhibition (Takakusaki et al., 2001). First, the interneurons would be excited from the NRGc with a late latency, and a proportion of them would also receive monosynaptic EPSPs from the NRGc. Second, the interneurons would receive inhibition from volleys in FRAs. Third, the interneurons would include a particular group of Ib inhibitory interneurons. The present study revealed the presence of interneurons that satisfied the above criteria. As illustrated in Fig. 14B, such interneurons (n=39) were mainly located in the ventromedial part of grey matter (lamina VII). A considerable proportion of this cell group (n=35) was monosynaptically, as well as polysynaptically, excited from the NRGc. Ten of these cells received monosynaptic excitation from group I afferents (filled circles).

Discussion

The present study provided the following new findings. First, the interneurons which were proposed as mediators for a medullary-induced motor inhibition were located in the ventromedial portion of lamina VII. Second, interneurons in reflex pathways mostly receive postsynaptic inhibitory inputs from the NRGc. Third, a functional topography was observed in the interneuronal organization with respect to the medullary-induced motor inhibition. In the following discussion the functional role of the reticulospinal control of interneuronal systems is considered with respect to the regulation of movements.

Consideration of the experimental procedures

In the present study although the maximum stimulus strength was lower than 50 μA there was a need to consider the current spread of the stimuli. The current spread of the MRF stimuli has been described previously (Habaguchi et al., 2002; Takakusaki et al., 2001). Briefly, in an investigation by Hentall et al. (1984) which used rats, it was calculated that the current spread of stimuli with a strength of 50 μA was approximately 250 μm . This value would be appropriate for the present investigation if it is assumed that the density and membrane properties of the MRF neurons of rats and cats are similar. Moreover, based on the finding of Mitani et al. (1988) the cell density in the cat NRGc is estimated to be approximately 3000 per mm^2 . Consequently, if the volume of the MRF affected by the stimulation (50 μA) encompassed a sphere with a radius of approximately 250 μm , the stimuli could excite 150 neurons.

Nonetheless, the MRF stimuli may activate not only cell bodies but also fibers of passage. Because pontine reticulospinal fibers arising from the nucleus reticularis pontis oralis descend in the NRGc at the level of the medulla (Matsuyama et al., 1993) MRF stimuli could possibly activate pontine reticulospinal fibers coursing through the NRGc. Cells which were monosynaptically activated from the NRGc were located in the ventromedial portion of the grey matter (Fig. 13A) where axonal terminals of

reticulospinal neurons arising from the NRGc are abundantly distributed (Bausbaum et al., 1978; Matsuyama et al., 1988). This finding suggests that the effects of electrical stimulation could be due to activation of the reticulospinal neurons in the NRGc.

Organization of interneuronal systems mediating the generalized motor inhibition

We have shown that NRGc stimulation induced early and the late IPSPs in forelimb and hindlimb motoneurons (Habaguchi et al., 2002; Takakusaki et al. 2001). The late IPSPs were observed in most (98%) of the motoneurons (Fig.4Ab). We have suggested therefore, that neuronal mechanisms for evoking the late IPSPs are responsible for the generalized motor inhibition. Several studies have suggested that the early and late IPSPs in motoneurons are mediated by fast-conducting (Takakusaki et al., 1989, 1994) and slow-conducting (Habaguchi et al., 2002; Kohyama et al., 1998) reticulospinal neurons, respectively. We further demonstrated that fast-conducting fibers and slow-conducting fibers mainly descended through the ventral funiculus and the ventrolateral funiculus, respectively (Takakusaki et al., 2001). On the other hand, NRGc stimulation induced either excitatory or inhibitory effects on the interneurons. More than one-third of the cells did not respond to NRGc stimulation (Fig.4Aa). Moreover there was a clear topographical arrangement of the interneurons with respect to the NRGc input (Fig. 14A). These findings suggest that the descending volley from the NRGc simultaneously controls the excitability of various categories of interneurons so that it produces a generalized motor inhibition.

Three matters may now be discussed:

(1) Are there interneurons mediating the reticulospinal inhibition of motoneurons?

The present study revealed the presence of inhibitory premotor interneurons which we have proposed as those which mediate the reticulospinal inhibition of motoneurons (Takakusaki et al., 2001). These interneurons were mainly located in the lamina VII of Rexed (Fig.14B). The optimal stimulus sites for evoking monosynaptic

excitation, and the sites for evoking late excitation in the interneurons, were almost the same (Fig.7). Thus both fast- and slow-conducting fibers may converge on the same interneurons that mediate the motor inhibition. Moreover the time course of the EPSPs in the above interneurons was similar to that of the NRGc-induced IPSPs in the interneurons (Table 2) and in the motoneurons (Habaguchi et al., 2002; Takakusaki et al., 2001). These findings suggest that interneurons that mediate reticulospinal inhibition of motoneurons may also inhibit interneurons interposed in reflex pathways. It is thus necessary to establish whether these interneurons provide postsynaptic inhibitory effects on both motoneurons and interneurons. As shown in Fig. 12B, a majority of interneurons in this category had a firing rate of less than 1 Hz (Fig. 12B), indicating that the excitability of the inhibitory premotor interneurons might be suppressed in the decerebrate animal.

(2) Reticulospinal control of interneurons in reflex pathways

Voluntary muscle contractions require parallel and balanced excitation of α - and γ -motoneurons and corresponding reciprocal Ia interneurons (α - γ linkage in reciprocal Ia inhibition, Hongo et al., 1969; Hultborn et al., 1976). Renshaw cells affect all of these neurons (Hultborn et al., 1976). The group II interneurons which are located in the intermediate region and ventral horn (lamina VI–VIII) are considered to be the last order interneurons (Cavallari et al., 1987) and have strong excitatory effects on both α - and γ -motoneurons (Appelberg et al., 1983). The medullary inhibitory inputs to these last-order interneurons, which are in parallel to both α - and γ -motoneurons (Takakusaki et al., 2001), may simultaneously alter the excitability of these neurons, and therefore play an important role in the regulation of postural muscle tone.

Among the group I (Ib) interneurons only those mediating postsynaptic inhibition of motoneurons (Hongo et al., 1983; Brink et al., 1983a) and presynaptic inhibition (Rudomin et al., 1987) have been identified. The group I interneurons were located in the intermediate region and were excited by volleys in FRAs. In the present study NRGc stimulation either inhibited or did not affect the activity of these cells. On the other hand,

lamina VII group I interneurons were excited by the NRGc. The existence of such a different population of group I interneurons is interesting with respect to the control of postural muscle tone. Group I interneurons are co-activated by tendon organs, a number of primary afferents from muscles of different joints, and by descending tract fibers. Consequently, during movements they could adjust and co-ordinate the activity of muscles operating different joints (see Jankowska, 1992). Thus, an interaction between group I interneurons in the intermediate region and those in lamina VII may be important for regulating the level of postural muscle tone. This regulation would occur via mutual inhibitory connections between the different populations of group I interneurons (Brink et al., 1983b).

(3) Interaction between the reticulospinal system and FRA system

A convergence of supraspinal descending tracts and FRA pathways on interneurons is required to mediate centrally-initiated complex motor synergies. For example, the rubrospinal and corticospinal tracts facilitate FRA pathways (Hongo et al., 1969, 1972; Hultborn et al., 1976; Jankowska et al., 1976; Lundberg et al., 1962; Lundberg and Vooehoeve, 1962) so that these tracts can produce fine movements (see Jankowska, 1992). Signals from the midbrain locomotor region also excite group II interneurons (Edgley et al., 1988; Schefchyk et al., 1990) and induce rhythmic firing of reciprocal Ia interneurons (Carol and Jordan, 1987; Feldman and Orlovsky, 1975) and Renshaw cells (Carol and Jordan, 1987; McCrea et al., 1980; Noga et al., 1987) to evoke locomotor movements. Lundberg et al. (1987b) have hypothesized that centrally-induced movements are mediated by subsets of excitatory group II interneurons with input from the muscles which are to be activated. Subsets of inhibitory group II interneurons are then used to prevent contractions of the other muscles. This hypothesis is particularly interesting with respect to the reticulospinal control of movements. In the present study interneurons excited from volleys in FRAs were inhibited by NRGc stimulation, while those inhibited by FRAs were excited from the NRGc (Fig. 12A). This observation

indicates that the excitability of the interneurons can be appropriately regulated by a counteraction between the medullary reticulospinal system and the FRA system. The counteraction may be beneficial to evoke purposeful movements in addition to adjustment of the level of muscle tone.

Functional implication of the inhibitory reticulospinal system

Postural muscle tone is completely diminished during rapid eye movement (REM) sleep. In this condition the spinal reflexes and the excitability of motoneurons are profoundly depressed (Glenn and Dement 1981a, 1981 b, 1981c; Morales and Chase, 1978). But supraspinal neurons arising from the cerebral cortex, the red nucleus, and the vestibular nucleus have been shown to be active during REM sleep (Arduini et al., 1963; Bizzi et al., 1964). Chase et al. (1986) demonstrated that stimulation of the NRGc induced late IPSPs in hindlimb motoneurons during a period of REM sleep of unrestrained cats. When the animals were awake stimulation induced a mixture of EPSPs and IPSPs. The time course of the REM-specific IPSPs was similar to that of the NRGc-induced IPSPs in motoneurons (Takakusaki et al., 2001) and that of the NRGc-induced IPSPs in interneurons which were observed in the present study. We consider that the reticulospinal system from the NRGc postsynaptically inhibits motoneurons and excitatory interneurons that are interposed in reflex pathways. This system also prevents the excitation of these neurons from other descending systems. This then contributes to a generalized motor inhibition during REM sleep. It is thus interesting to examine whether lamina VII interneurons identified in the present study contribute to the postsynaptic inhibitory process that acts during the period of REM sleep.

We have shown that the basal ganglia efferents to the pontomedullary reticular formation via the pedunculo-pontine tegmental nucleus (PPN) controls postural muscle tone of the decerebrate cat (Takakusaki et al., 2003). The PPN receives a dense GABAergic input from the substantia nigra pars reticulata (SNr), one of the output nuclei

in the basal ganglia (Beckstead et al., 1979; Moriizumi et al., 1988; Rye et al., 1987; Span and Grofova, 1991). It is possible therefore that the medullary reticulospinal system is involved in basal ganglia related movements. It follows that the basal ganglia-brainstem system can be particularly important in relation to the control of postural muscle tone. For example, dystonia (abnormal muscular tonus) is one of symptoms of basal ganglia disease. The GABAergic output from the basal ganglia is considered to increase in Parkinson's disease (Alexander and Crutcher, 1990; DeLong, 1990). We have postulated that the excessive GABAergic inhibitory effects upon the PPN greatly reduce the activity of the medullary reticulospinal system. This results in an increased muscle tone (hypertonus) in this disease (Takakusaki et al., 2003). Delwide et al. (2001) have suggested that in Parkinson's disease there is a dysfunction in the medullary reticulospinal tract arising from the NRGc and Ib inhibitory pathway. Elucidation of the activity of the reticulospinal-interneuronal systems during movements would enable a better understanding of the pathophysiological mechanisms of motor disturbances in basal ganglia disorders.

Conclusions

The present paper directly examined spinal interneurons and has provided solid evidence from the suggestion that the medullary reticulospinal tract controls posture muscle tone and movements by regulating the excitability of not only motoneurons but also interneurons in transmission of reflex pathways. A group of interneurons, which are located in the lamina VII and VIII, possibly provide postsynaptic inhibitory effects upon both the motoneurons and interneurons.

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Figure legends

Figure 1. The experimental set-up and optimal stimulus sites for suppression of postural muscle tone of the decerebrate cat.

(A). Experimental set-up. See method section for a detailed explanation. (B). Optimal stimulus sites (n=27) in coronal sections at the levels of P 8.5, P 9.2 and P 10.0. The sites were located in the medial part of the medial reticular formation corresponding to the nucleus reticularis gigantocellularis (NRGc). CDPs, cord dorsum potentials, NRMc, the nucleus reticularis magnocellularis; NRPv, the nucleus reticularis parvocellularis; MLF, the medial longitudinal fasciculus; IO, inferior olive.

Figure 2. Possible neuronal mechanisms of a generalized motor inhibition that we have previously proposed (Takakusaki et al., 2001).

A. The medullary induced effects are exerted in parallel on α - and γ -motoneurons, and on interneuronal transmission in reflex pathways to the motoneurons. B. The inhibitory effects upon the motoneurons could be mediated by interneurons which were characterized by early and late excitation from the MRF and inhibition from flexor reflex afferents. A part of the interneurons also received monosynaptic excitation from group I muscle afferents, i.e., the interneurons possibly included a group of Ib inhibitory interneurons. α , α -motoneurons; γ , γ -motoneurons; E, excitatory interneurons; I, inhibitory interneurons; FRAs, flexor reflex afferents; Gr. I, group I muscle afferents; R, Renshaw cells; MNs, motoneurons; INs, interneurons.

Figure 3. Characteristics of the medullary-induced effects upon interneurons.

A. Intracellular and extracellular recordings of the same interneuron. The traces illustrate (from upper to lower) intracellular potentials, extracellular potentials, and CDPs. Upward arrows indicate the NRGc stimuli. Upward and downward arrowheads indicate the onset

of the EPSPs and the action potentials, respectively. B and C. Intracellular recordings of EPSPs (B) and IPSPs (C) evoked from the NRGc. The upper and lower traces are intracellular potentials and CDPs, respectively. Each recording was an average of 10 sweeps. (a)~(e) Shape parameters of a late EPSP (B) and a late IPSP (C). They are indicated for the latency (a), peak latency (b), duration (c), half width (d) and amplitude (e). These shape parameters correspond to those in Table 2. The upward arrows indicate the NRGc stimuli. D. Frequency histograms of the segmental delay of early PSPs. (a) Segmental delay for early EPSPs. (b) Segmental delay for early IPSPs. Each triangle shows the mean value of the segmental delay of each potential. E. Frequency histograms of the segmental latency of action potentials with an early latency. Each triangle shows the mean value of the segmental latencies.

Figure 4. Late medullary stimulus effects on interneurons.

A. (a). Late effects from the NRGc on interneurons in the lower lumbar segments. Fifty-five (28.8%) and 67 (35.1%) cells received late excitatory and inhibitory inputs from the NRGc. However 69 (36.1%) did not respond to the NRGc stimulation in the late latency. (b) On the basis of our previous studies (Habaguchi et al., 2002; Takakusaki et al., 2001), stimulation of the NRGc induced late IPSPs in most hindlimb motoneurons (n=212/216, 98.1%) in acute decerebrate cats. B. Late effects from the NRGc on selected groups of interneurons. See text for further explanation.

Figure 5. Intracellular recording of group I interneurons excited from the medulla. Upper and lower traces indicate intracellular potentials and CDPs, respectively. A. Effects from the NRGc in early (a) and late (b – c) latencies with a stimulus intensity of 30 μ A. Action potentials were not observed with single pulses (b) but were generated by train pulses of stimuli (c) applied to the NRGc. Effects from group I muscle afferents (d – h) and a cutaneous afferent (i). Absence of recurrent IPSPs (j). A voltage calibration in (j) also

applies to (a – i). B. Effects from the NRGc of single pulses (a) and three trains of pulses (b) with a stimulus intensity of 30 μ A. Hyperpolarizing current of 2.0 nA was injected into the cell to stop firing during this recording (b). Effects from group I muscle afferents (c – f) and a cutaneous afferent (g). Absence of recurrent IPSPs (h). Open and filled arrowheads indicate the onset of the early EPSPs and first positive peak of a descending volley from the NRGc. The segmental delay was 0.7 ms in cell (A) and 0.5 ms in cell (B). A voltage calibration in (h) also applies to (a – g). C. Possible connections with these cells. Both cells received early (monosynaptic) and late EPSPs from the NRGc, monosynaptic group I muscle afferents and polysynaptic inhibition from volleys in FRAs. Cells (A) and (B) were recorded at 2.9 mm and 3.1 mm from the surface of cord dorsum, respectively.

Figure 6. Interneurons excited from the medulla and inhibited from flexor reflex afferents. Upper and lower traces indicate intracellular potentials and CDPs, respectively. A. Effects from the NRGc in early (a) and late (b) latencies with a stimulus intensity of 40 μ A. Effects from high threshold muscle afferents (c – e) and a cutaneous afferent (f). Open and filled arrowheads indicate the onset of the early EPSPs and first positive peak of a descending volley from the NRGc respectively. The segmental delay was 0.6 ms. Lower traces in (a) and (b) are extracellular potentials records from just outside the cell after recording. A voltage calibration in (f) also applies to (a – e). B. Effects from the NRGc in early (a) and late (b) latencies with a stimulus intensity of 40 μ A. Volleys in high threshold afferents did not evoke action potentials (d – f). Open and filled arrowheads indicate the onset of the initial spikes and first positive peak of a descending volley from the NRGc, respectively. The segmental latency to the firing was 1.0 ms. A voltage calibration in (f) also applies to (a – e). C. Possible neuronal connections with these interneurons.

Figure 7. Optimal stimulus sites for evoking excitatory effects upon interneurons.

A. Stimulus sites in the medulla at P 10 and recording sites of two interneurons at a lumbar segment of L6. B. Identification of an interneuron, of which activities were obtained from intra-axonal recording. This interneuron was excited in early (a) and late (b) latencies. However, conditioning volleys in FRA from LG-S muscles completely eliminated these excitatory effects (c and d). Voltage calibrations and time scales in (c) and (d) also apply to (a) and (b), respectively. C. Early (a) and late latency (b) responses following the stimuli applied to each site in (A). Lower traces are CDPs. Open and filled arrowheads in the second set of recordings indicate the onset of the initial spikes and first positive peak of a descending volley from the NRGc, respectively. The segmental latency of this cell was 1.2 ms. Single pulses (a) and train pulses (b) with an intensity of 30 μ A were applied to the medulla. Excitatory effects were evident by stimuli applied to the NRGc. D. Effects of medullary stimuli delivered at each site indicated in (A) upon intracellular activity of an interneuron which was monosynaptically excited from group I afferents. Upper and lower traces are intracellular potentials and CDPs, respectively. Twin pulses of stimuli were delivered at a strength of 30 μ A.

Figure 8. Medullary stimulus effects upon reciprocal Ia inhibitory interneurons.

A. Intracellular recordings of a Q-coupled reciprocal Ia interneuron. Responses from Q (a) and PBSt (b) Ia afferents. Recurrent IPSPs (upper) and their reversal (middle) by injection of hyperpolarizing current (c). Medullary-induced early and late IPSPs (upper) and their reversal (middle) by injection of hyperpolarizing current (d). Lower traces in (a) and (b), and lowermost traces in (c) and (d) are CDPs. Effects from high threshold muscle afferent (e), and its inhibition of the NRGc-induced IPSPs (f). A voltage calibration in (f) also applies to (a – e). B. Possible neuronal connections with an interneuron (A). C. Extracellular recordings of PBSt-coupled reciprocal Ia interneuron. Monosynaptic excitation from PBSt Ia afferent (a) and its inhibition from recurrent motor axon

collaterals (b). Reciprocal Ia inhibition from Q Ia afferent (c). Excitation from volleys in cutaneous afferent (d). Spontaneous firing (e) was inhibited by stimulating the NRGc (f). A voltage calibration in (a) also applies to (b – f).

Figure 9. Medullary inhibitory effects upon group I interneurons in the intermediate region.

A. Intracellular recordings from PBSt-coupled intermediate group I interneuron. Upper and lower traces are intracellular recordings and CDPs, respectively. Monosynaptic excitation from group Ia afferents from PBSt muscle (a – b). This cell lacked recurrent IPSPs (c), and received an excitation from volleys in cutaneous afferents (d). Spontaneous firing (f) was blocked by early and late IPSPs induced by stimulating the NRGc. A voltage calibration in (d) also applies to (a – c) and (e - f). B. Extracellular recording of MG-coupled intermediate Ia interneuron. Upper and lower traces are intra-axonal potentials and CDPs, respectively. Monosynaptic activation from group I afferents from the MG muscle (a – b). The monosynaptic activation was not blocked by ventral root stimulation (c), and excitation from volleys in FRAs (d). Spontaneous firing (f) was blocked by NRGc stimulation with a stimulus intensity of 50 μ A (e). A voltage calibration in (c) also applies to (a –b) and (d - f). C. Possible neuronal connections with intermediate group I interneurons.

Figure 10. Medullary inhibitory effects upon a Renshaw cell.

A. Upper and lower traces are intracellular recording and CDPs, respectively. (a) and (b) Burst firing of a Renshaw cell with different time scales following the stimulation of the ventral roots (L7 and S1). (c) Early and late IPSPs evoked by 3 trains of NRGc stimulation with an interval of 2 ms. When NRGc was stimulated by 3 trains with an interval of 10 ms, the amplitude of the early IPSPs was increased as the number of stimulus pulses was increased (d). A recording during the period which is denoted by dashed bar is shown in

(e) with an expanded time scale. Upward arrows indicate NRGc stimulation. Filled and open arrowheads indicate the onset of the descending volley and the onset of the early IPSPs. The segmental delay was 1.4 ms. Voltage calibrations in (b) and (e) also applies to (a) and (c – d), respectively. B. Possible neuronal connections with Renshaw cells.

Figure 11. Medullary inhibitory effects upon interneurons excited from FRAs.

Upper and lower traces are intracellular recording and CDPs, respectively. A. A lack of monosynaptic excitation from group I muscle afferent (a). Polysynaptic excitation from high threshold muscle afferents (b, c) and cutaneous afferents (d). The NRGc-induced IPSPs (upper traces) and their reversal (middle traces) by hyperpolarizing current injection (e – f). Records (e) and (f) are shown with different time scales. Downward and upward arrowheads in (f) are onset of the IPSPs and the onset of the descending volley from the NRGc, respectively. Voltage calibrations in (d) and (f) also applies to (a – c) and (e), respectively. B. Excitation from cutaneous afferent from the SP nerve (a). The NRGc-induced IPSPs (b). Volleys in cutaneous afferents from SP (c) and Saphe (e) attenuated the NRGc-induced IPSPs. Responses from volleys in Saphe nerve (d). A voltage calibration and time scale in (a) also applies to (b – e). C. Excitation from high threshold muscle afferents (a, b). The NRGc-induced IPSPs (c). Volleys in high threshold muscle afferents blocked the NRGc-induced IPSPs (d). A voltage calibration in (d) also applies to (c). D. Possible neuronal connections with interneurons excited from FRAs.

Figure 12. Relationship between late medullary effects and actions from the FRAs (A) and spontaneous firing rate (B).

A. A comparison of late NRGc effects and actions from the FRA in the same interneurons which were recorded intracellularly (n=55). Proportions of cells excited (open), inhibited (filled) and with mixed excitatory-inhibitory FRA effects (hatched) are indicated. B. A comparison of late NRGc effects and spontaneous firing rate in the same interneurons

which were recorded extracellularly (n=136). Cells having firing rates of 1–10 Hz and those with more than 10 Hz are illustrated by hatched and filled symbols, respectively.

Figure 13. Location of interneurons in the lower lumbar segments in relation to the medullary stimulus effects.

A. Locations of 61 interneurons which received monosynaptic excitation from the NRGc. They were recorded from the ventromedial portion of grey matter corresponding to lamina VII and VIII of Rexed. B. Location of 55 interneurons which received late excitation from the NRGc. C. Sixty-seven interneurons which received late inhibition from the NRGc were located in dorsal horn, intermediate region and ventral horn of the grey matter. D. Sixty-nine interneurons which lacked the late NRGc effects were distributed in the dorsal horn and intermediate region. Filled and open circles indicate interneurons with and without monosynaptic excitation from group I muscle afferents, respectively. Intracellular and extracellular recordings were made from interneurons denoted by large and small symbols, respectively.

Figure 14. Organization of spinal interneurons with respect to the medullary-induced generalized motor inhibition. A. Topographical arrangements of interneurons and motoneurons in relation to the late NRGc effects. Interneurons receiving the late excitation were located in the ventromedial part of the grey matter (black). Interneurons receiving the late inhibition were dorsoventrally distributed in the grey matter (grey). Unresponsive cells were mainly located in the dorsal half of grey matter (hatched). B Location of 39 interneurons which were previously proposed as those mediating the medullary-induced motor inhibition (Takakusaki et al., 2001). Filled (n=29) and open (n=10) circles indicate interneurons with and without monosynaptic excitation from group I muscle afferents, respectively. Large and small symbols represent interneurons which were recorded intracellularly and extracellularly, respectively. See text for further explanation.

Table 1. Combination of the early and late postsynaptic potentials in interneurons

		Late PSPs			
		EPSP	IPSP	None	Sum
Early PSPs	EPSP	15	9	4	28
	IPSP	0	9	3	12
	None	5	7	3	15
Sum		20	25	10	55

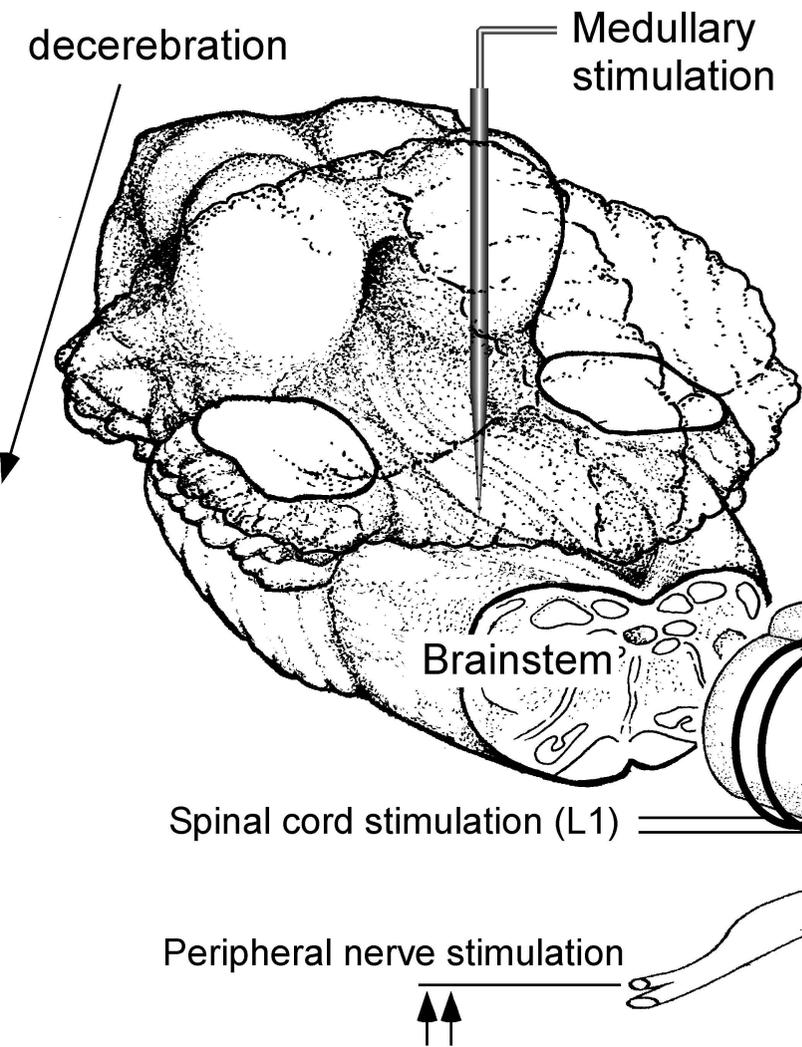
We judged the existence of the PSPs if they had an amplitude more than 1.0 mV at the resting membrane potentials in each interneuron following the NRGc stimuli (3–5 pulses, with a duration of 0.2 ms and intensity of 20–50 μ A).

Table 2. Shape parameters of the medullary-induced late postsynaptic potentials in interneurons.

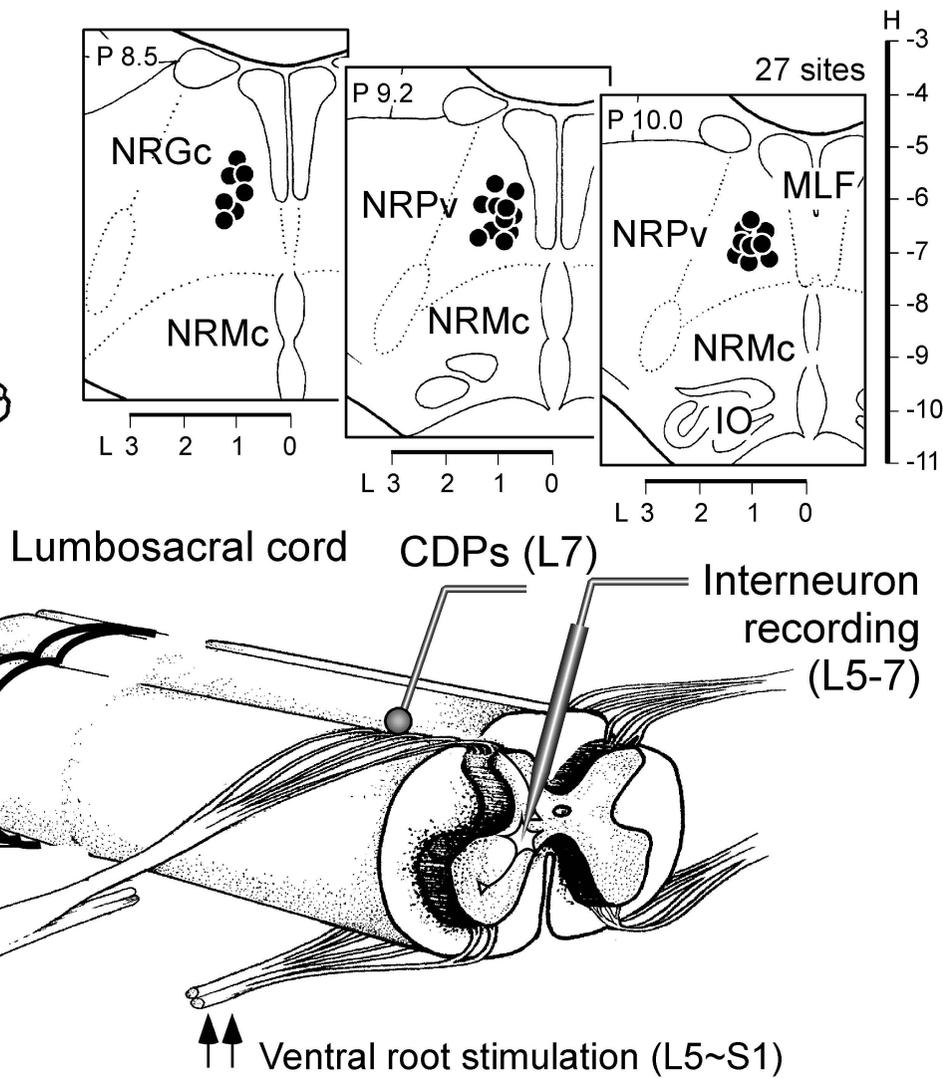
Shape parameters	Late EPSPs (n=18)	Late IPSPs (n=25)
a; latency (ms)	29.1 ± 4.0	33.6 ± 5.4
b; peak latency (ms)	50.3 ± 6.0	52.7 ± 7.1
c; duration (ms)	62.5 ± 11.8	46.0 ± 7.1
d; half width (ms)	36.6 ± 5.3	26.6 ± 5.5
e; amplitude (mV)	3.6 ± 1.3	7.6 ± 4.5

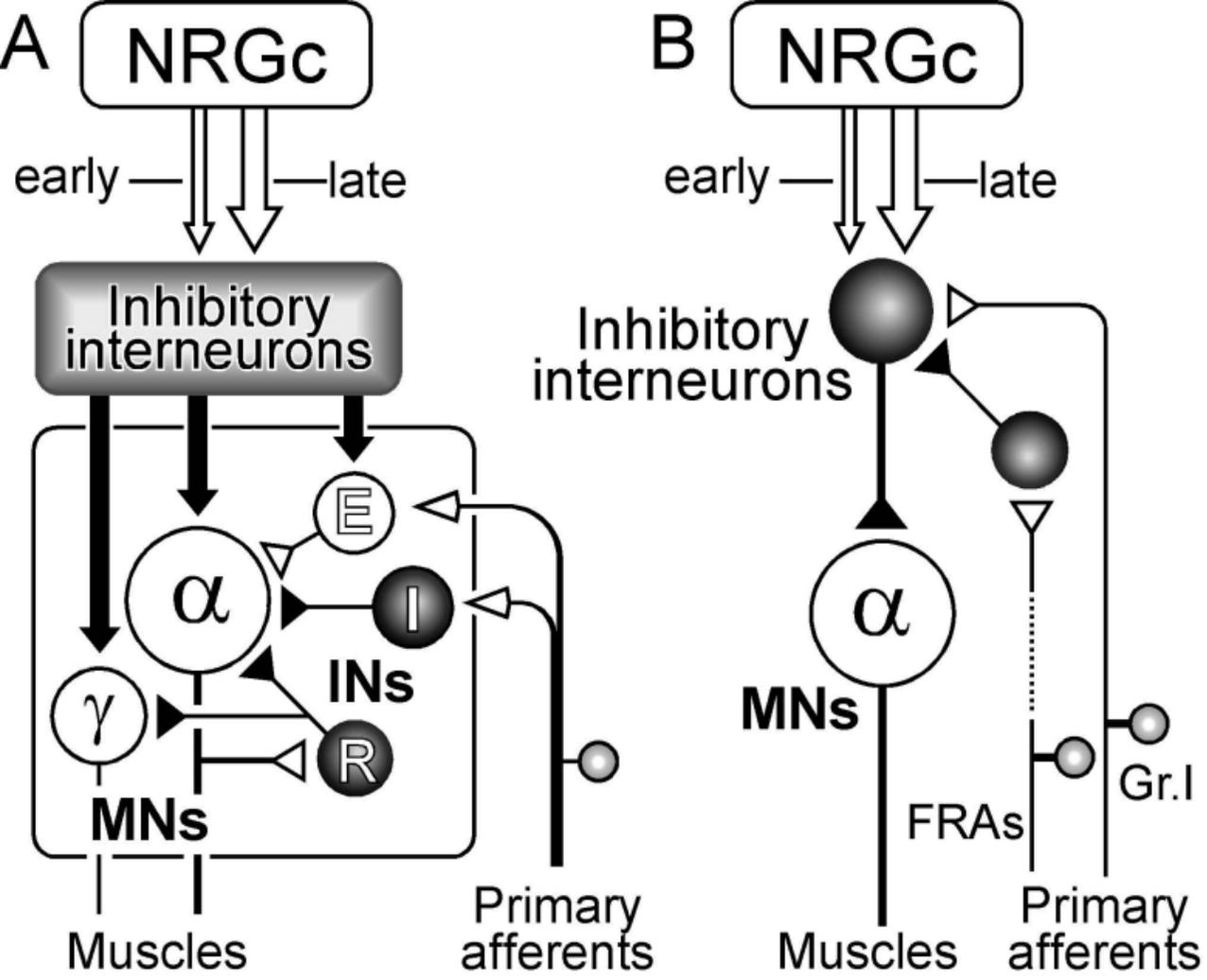
The shape parameters correspond to those in Fig. 3C and D. Each value (Mean ± Standard deviation) was obtained from 43 interneurons which were intracellularly recorded.

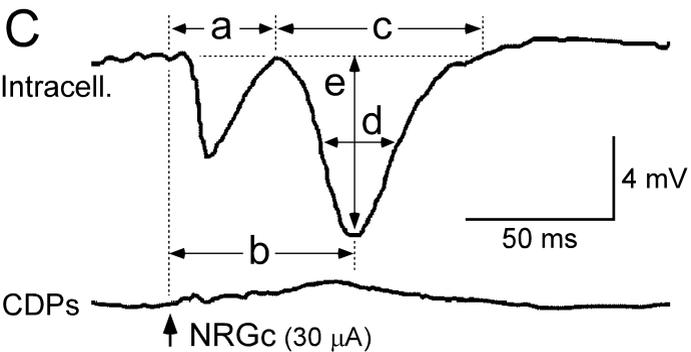
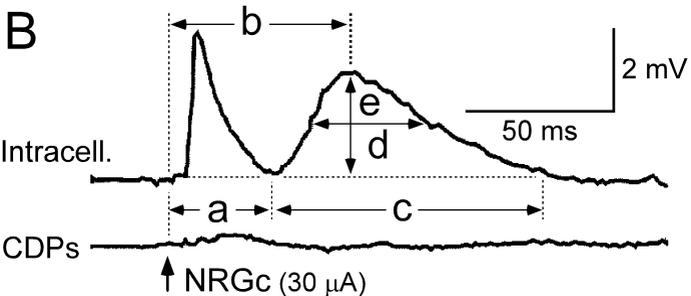
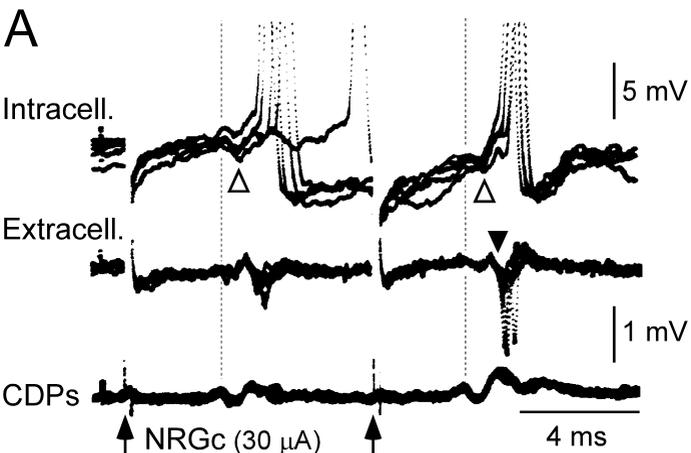
A Experimental set up



B Optimal sites for motor inhibition

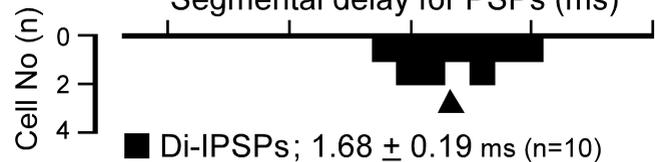
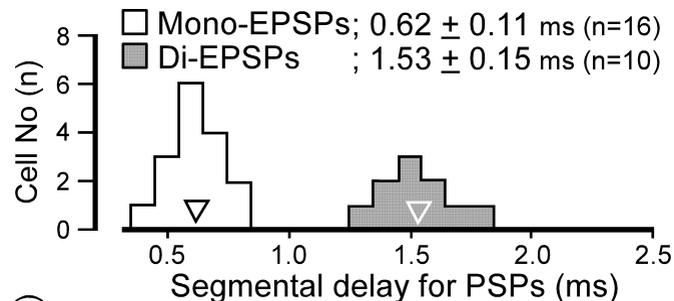






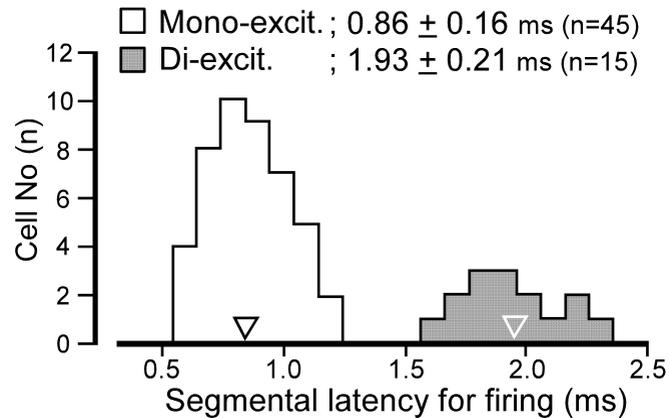
D Intracellular recording

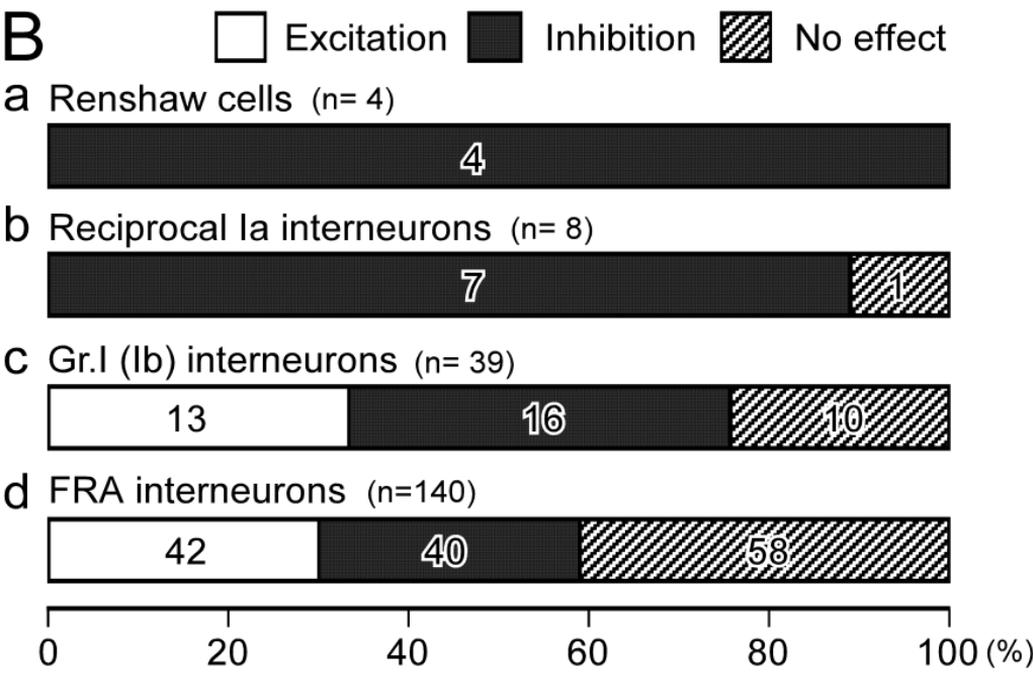
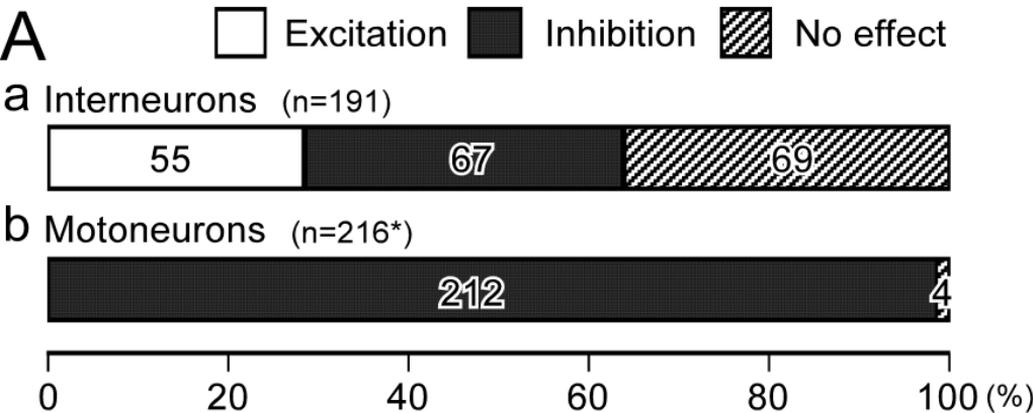
a early EPSPs

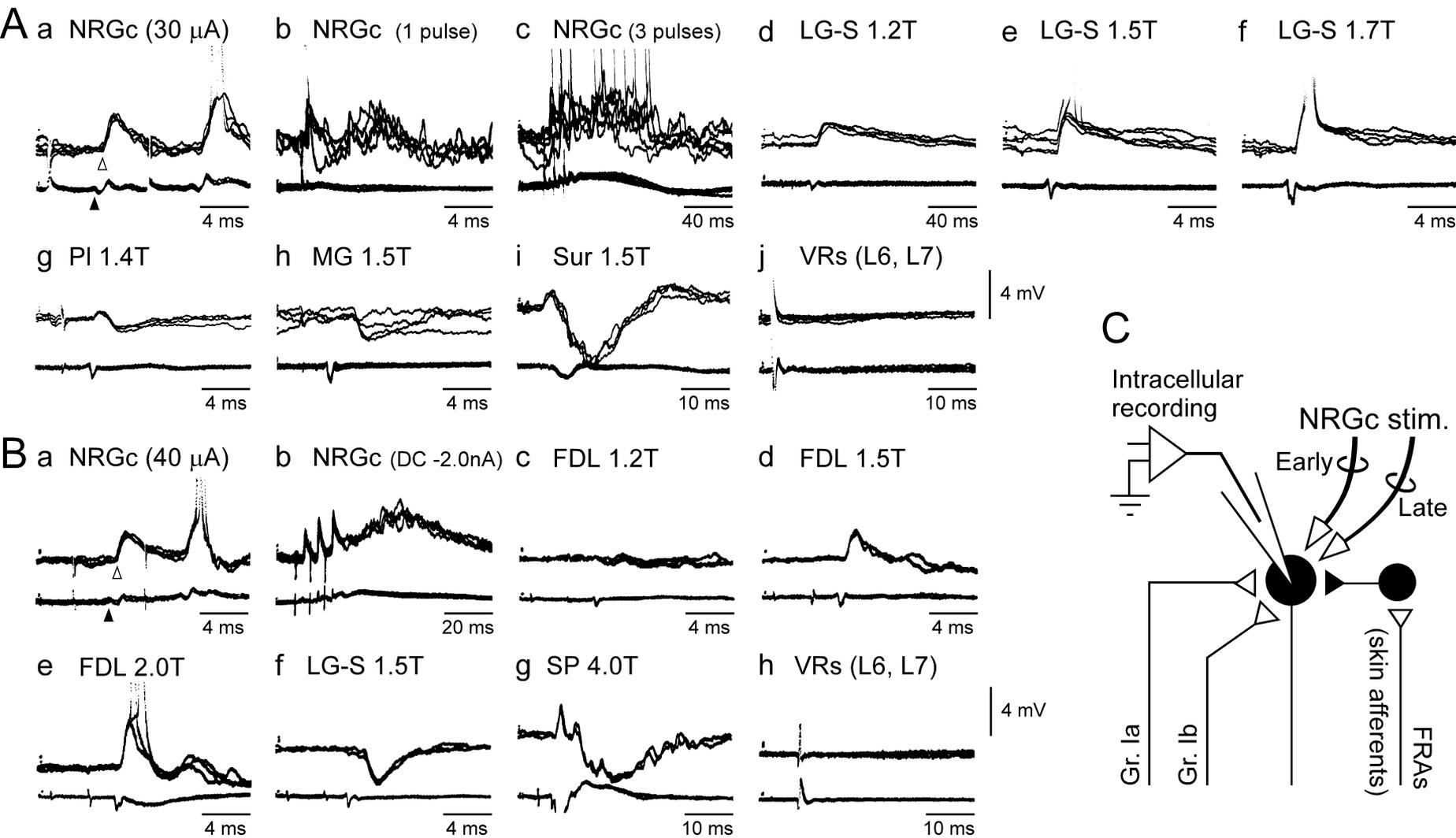


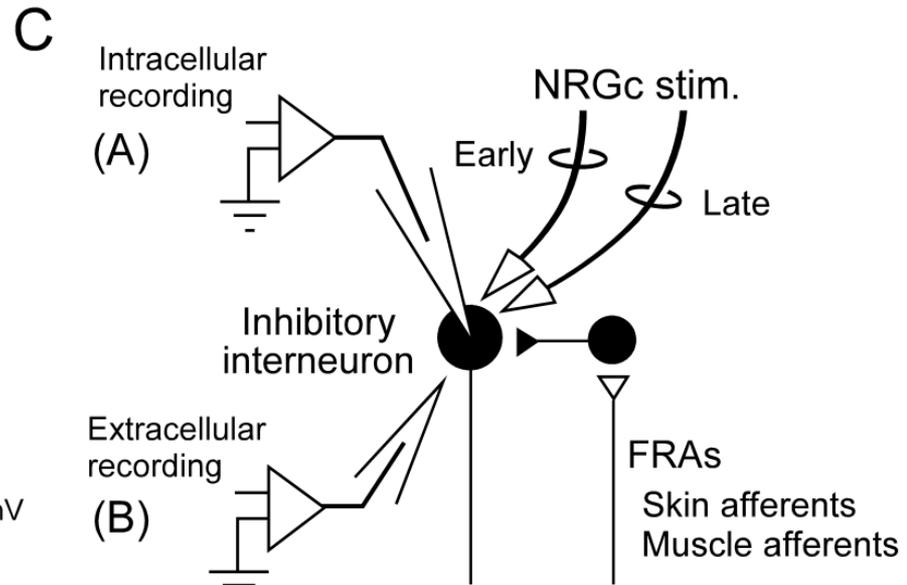
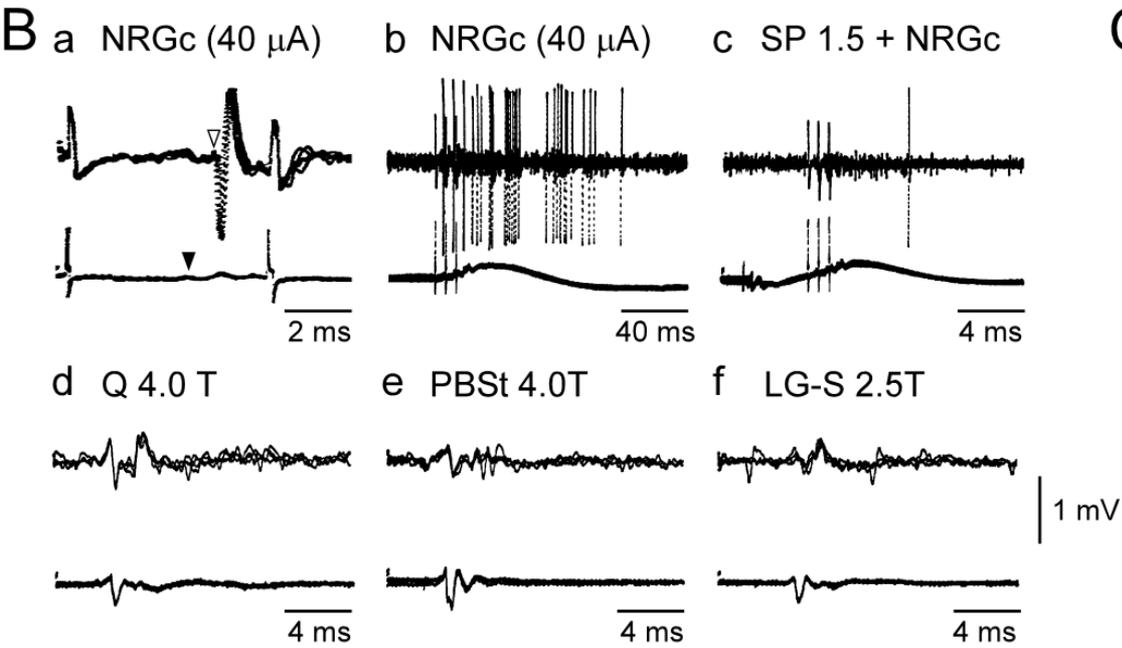
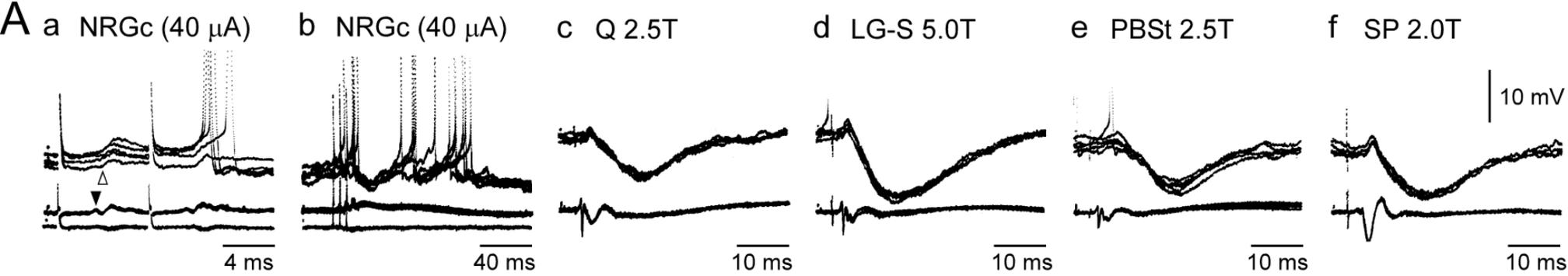
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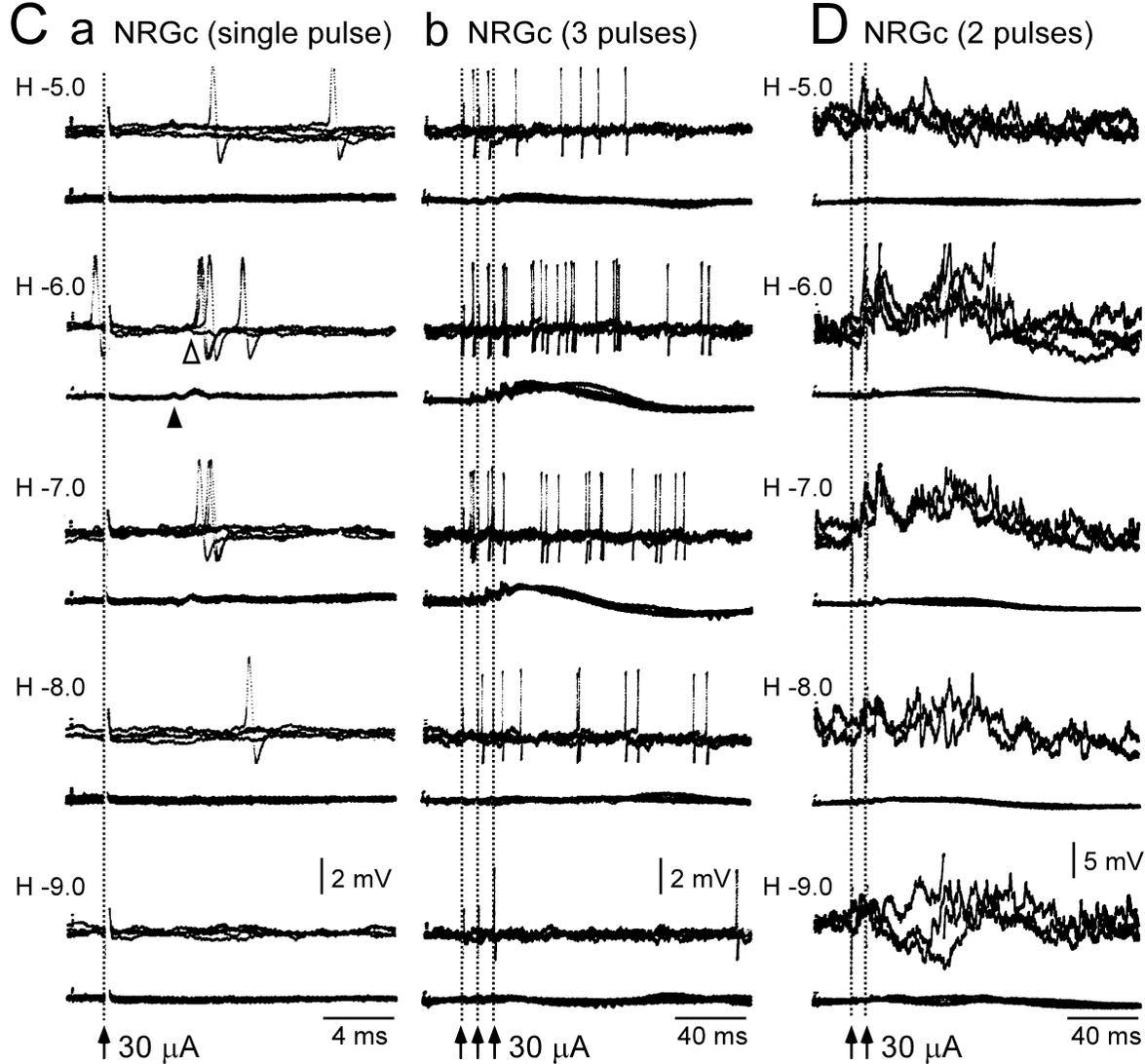
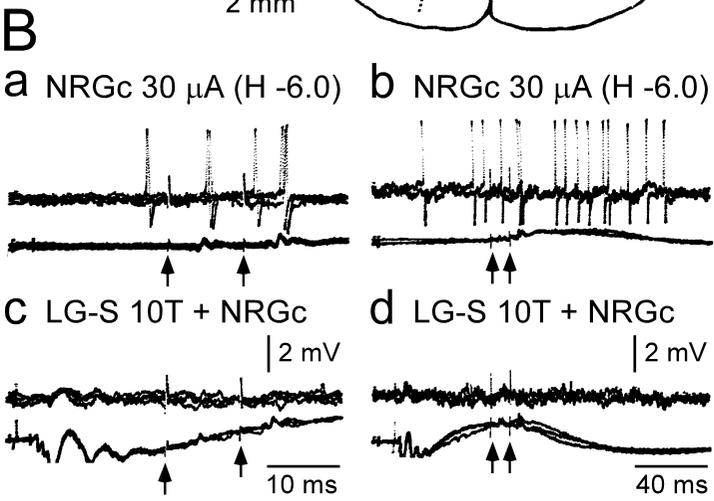
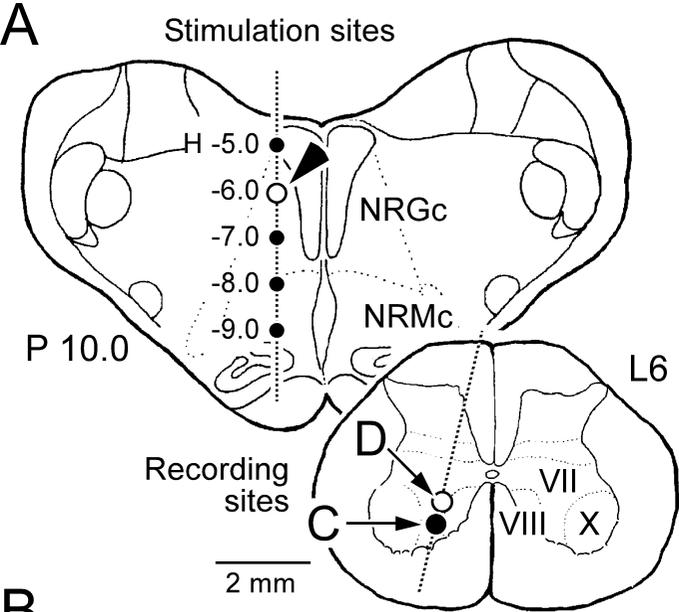
E Extracellular recording

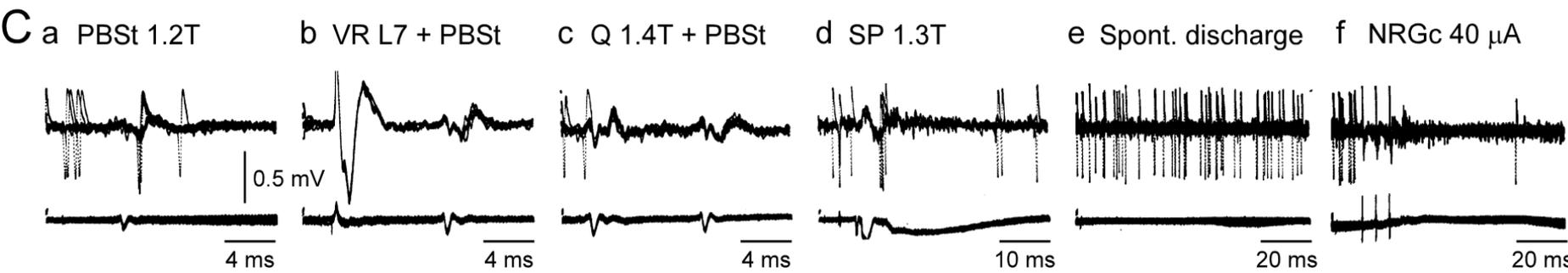
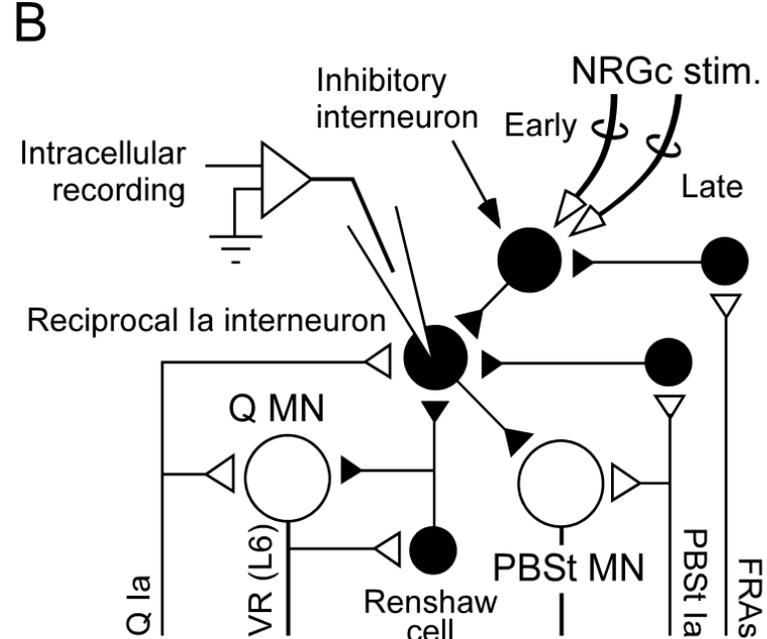
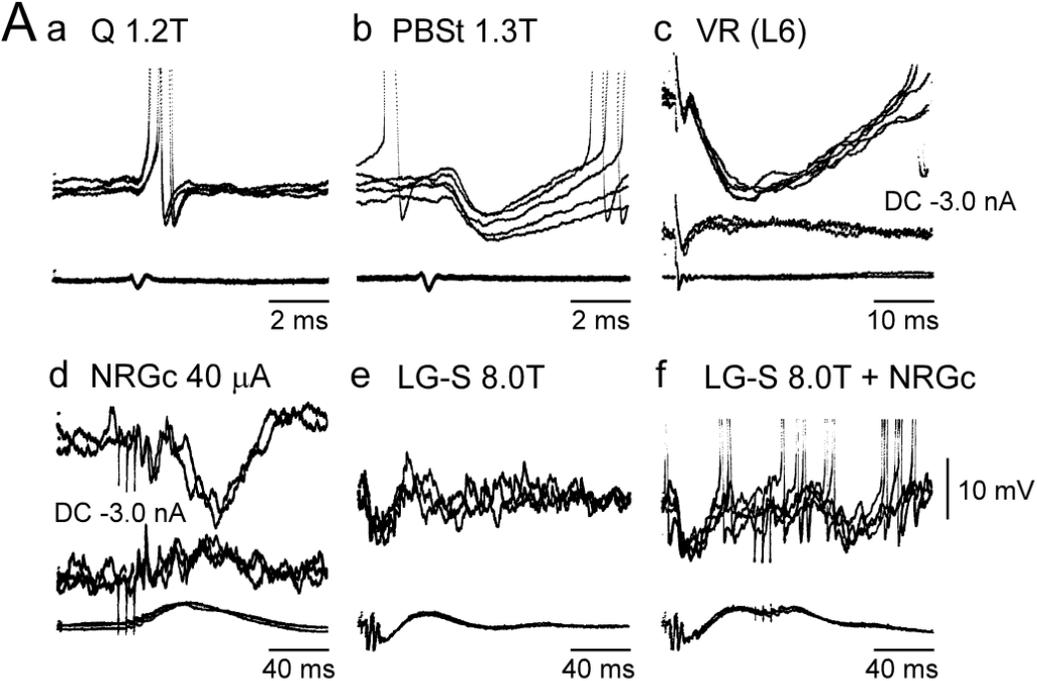


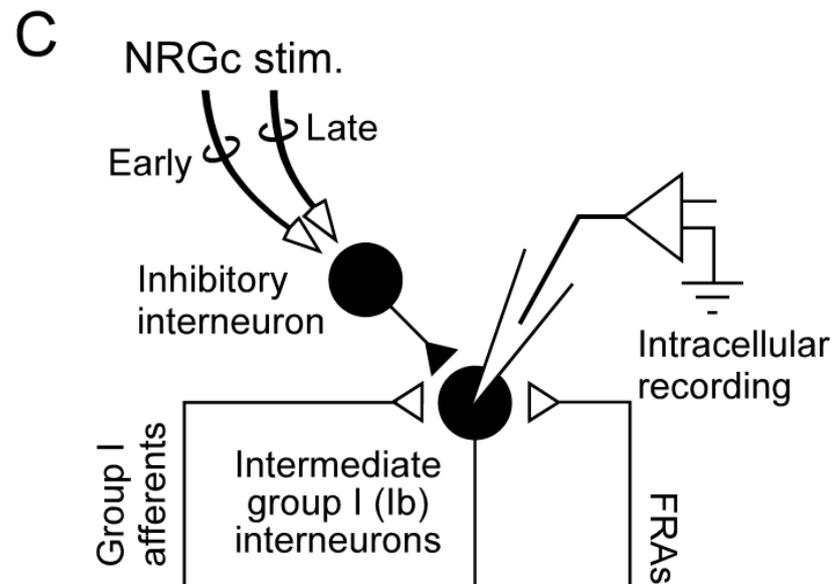
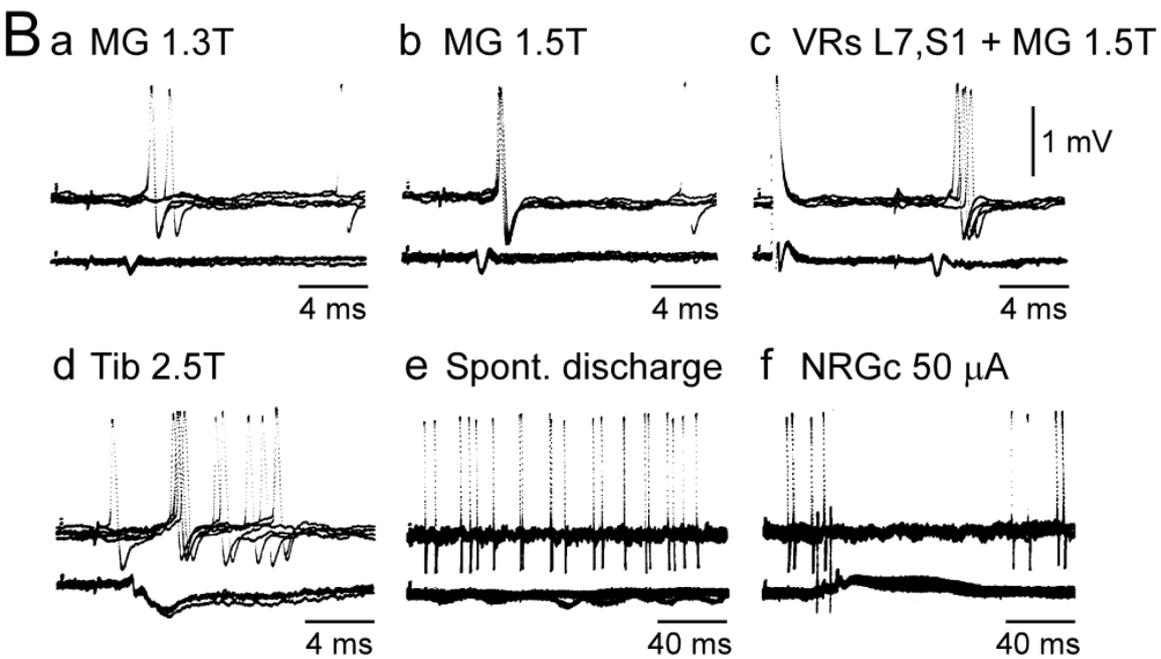
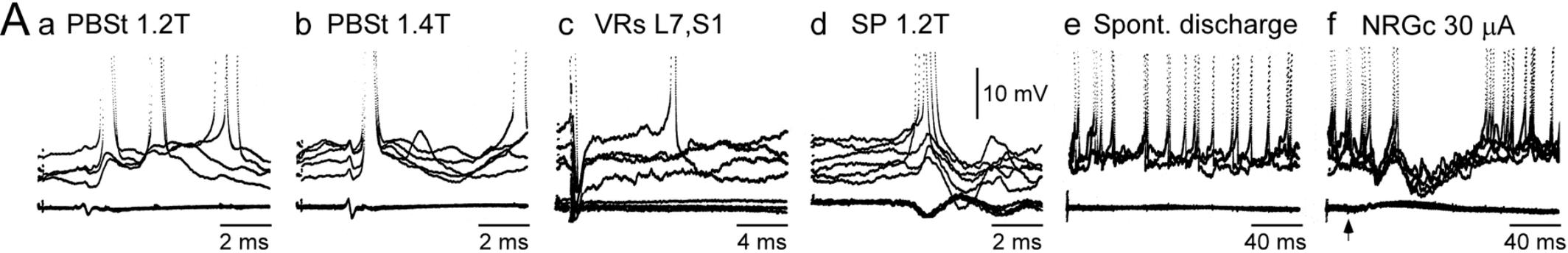


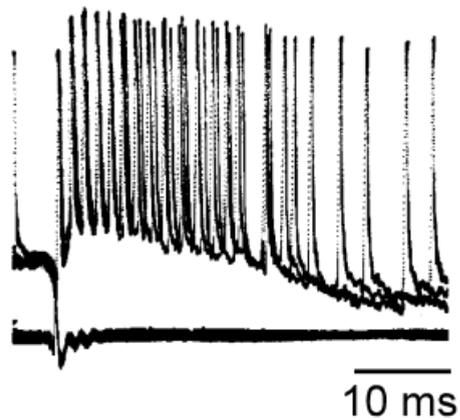
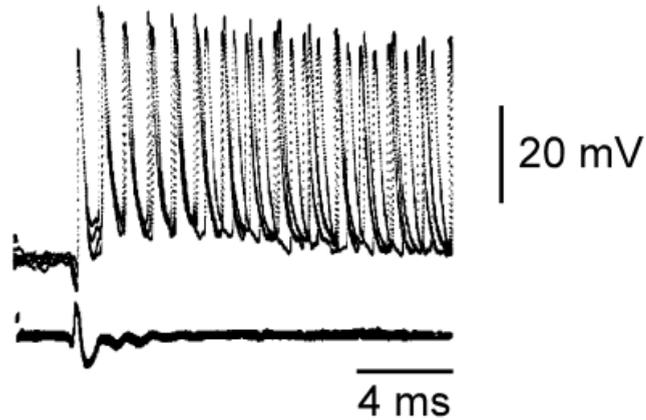
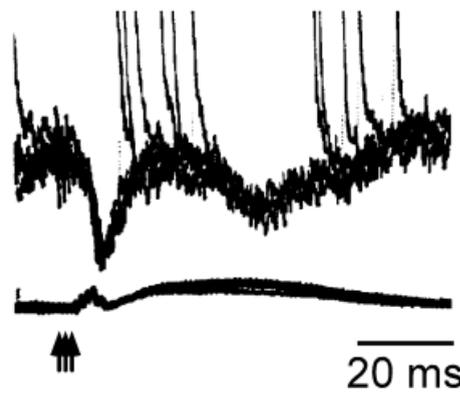
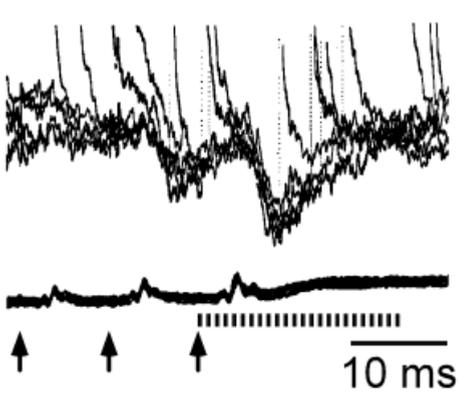
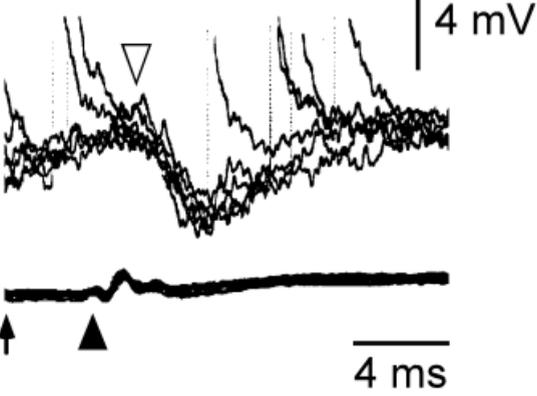
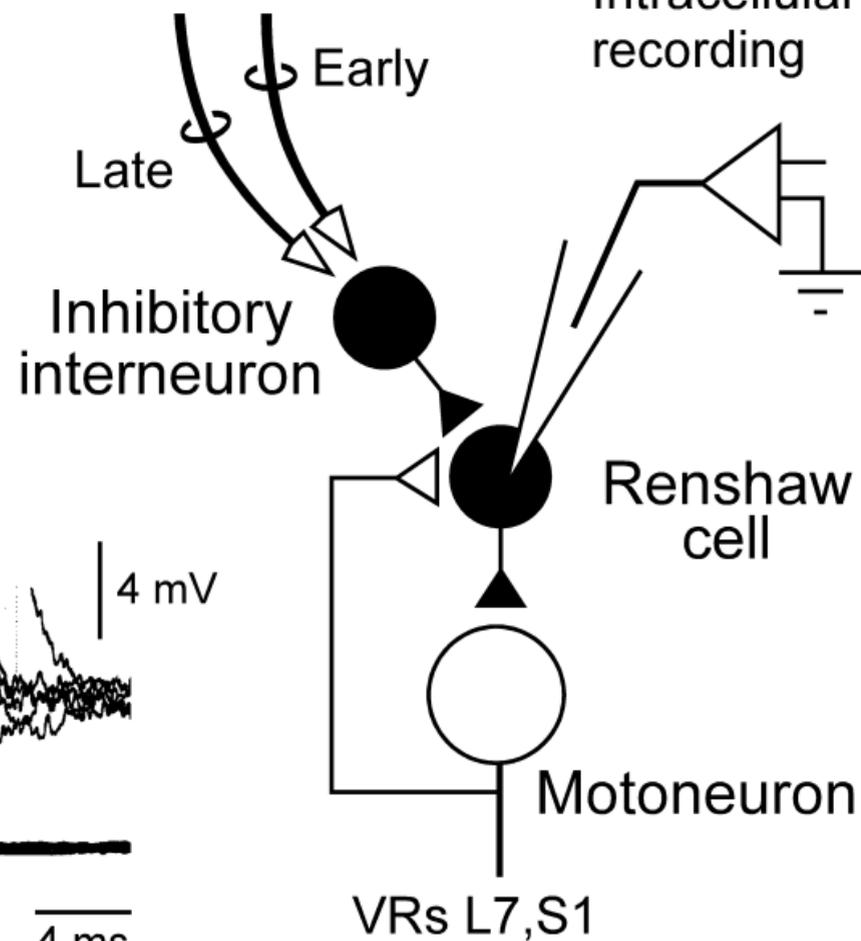


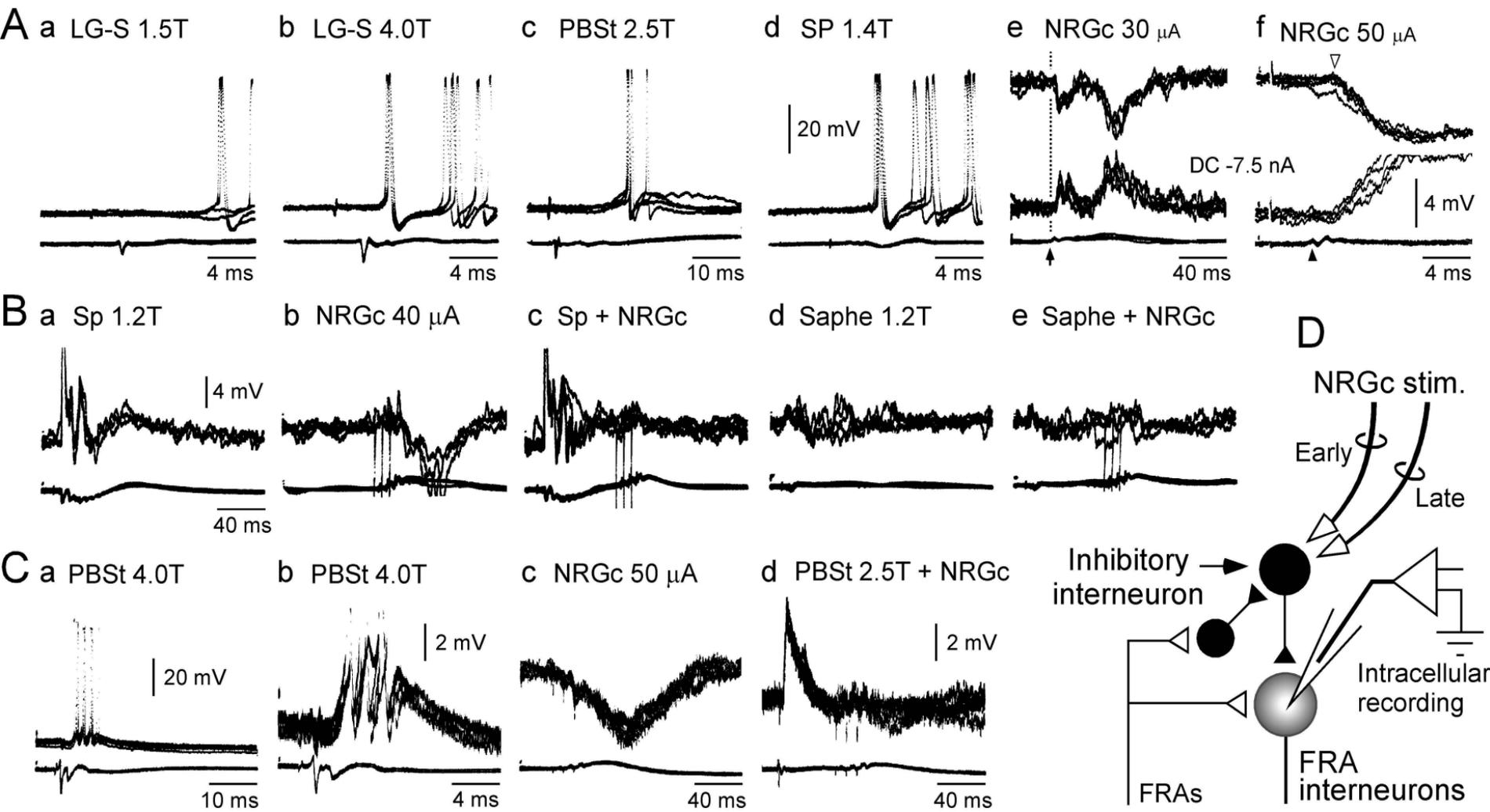


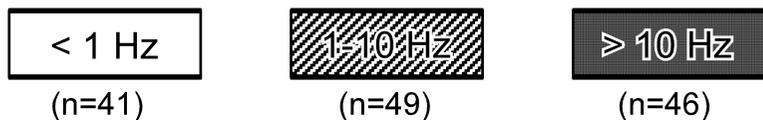
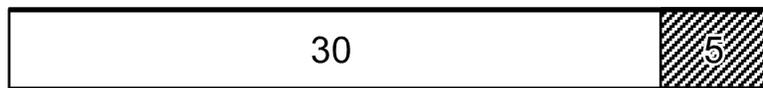


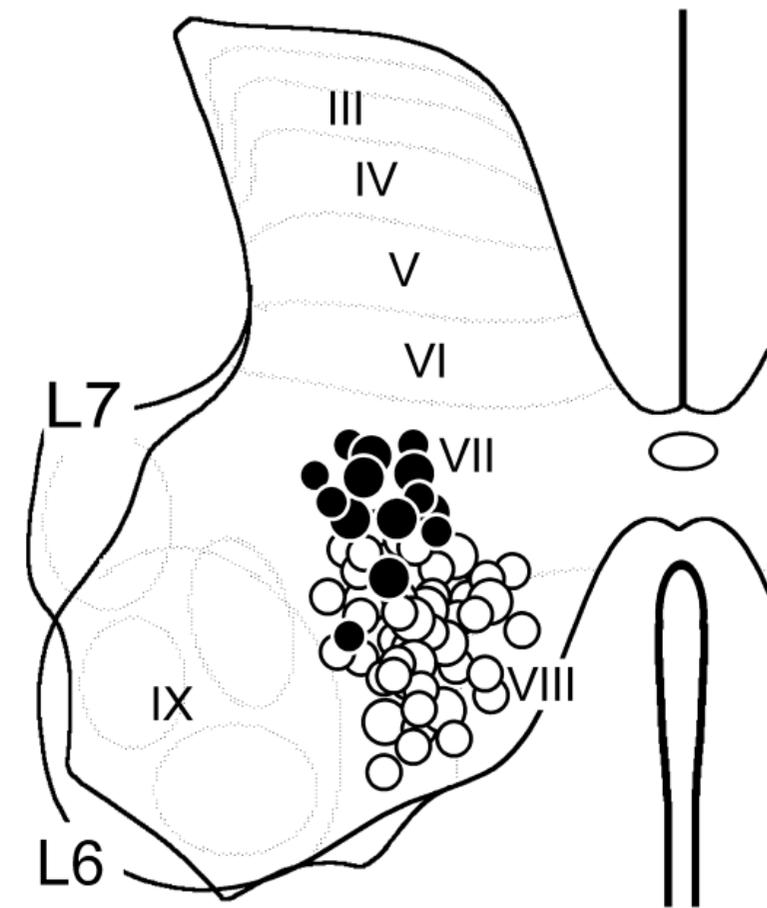
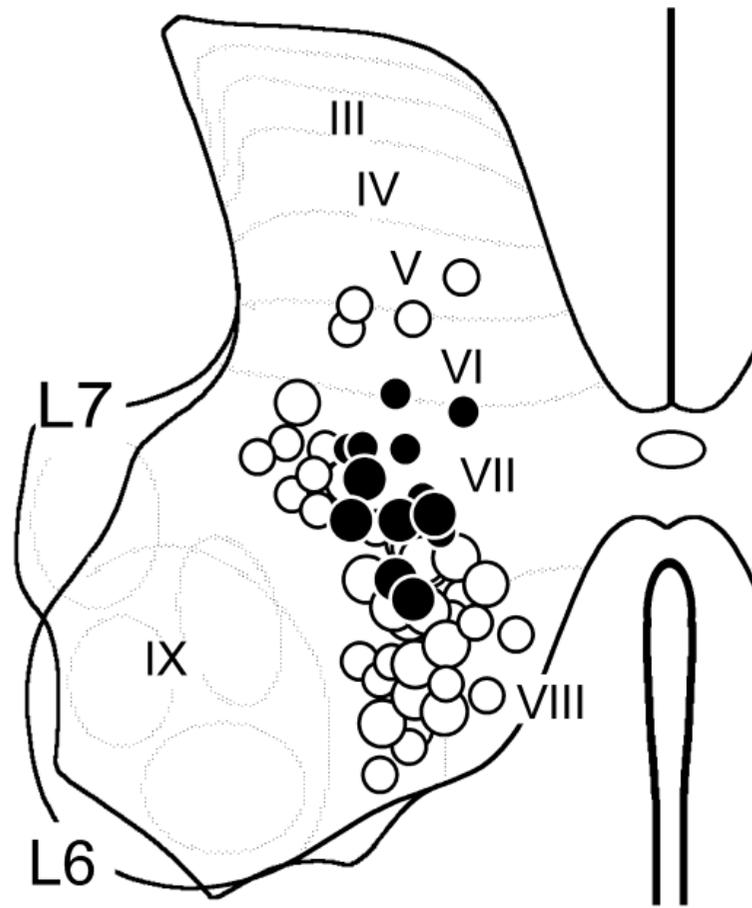
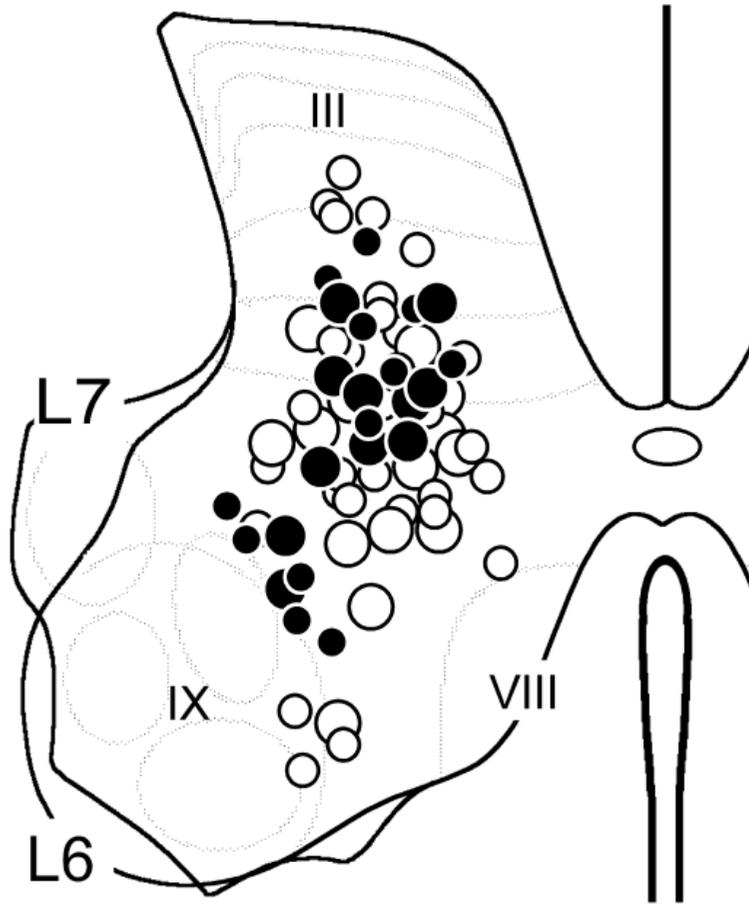
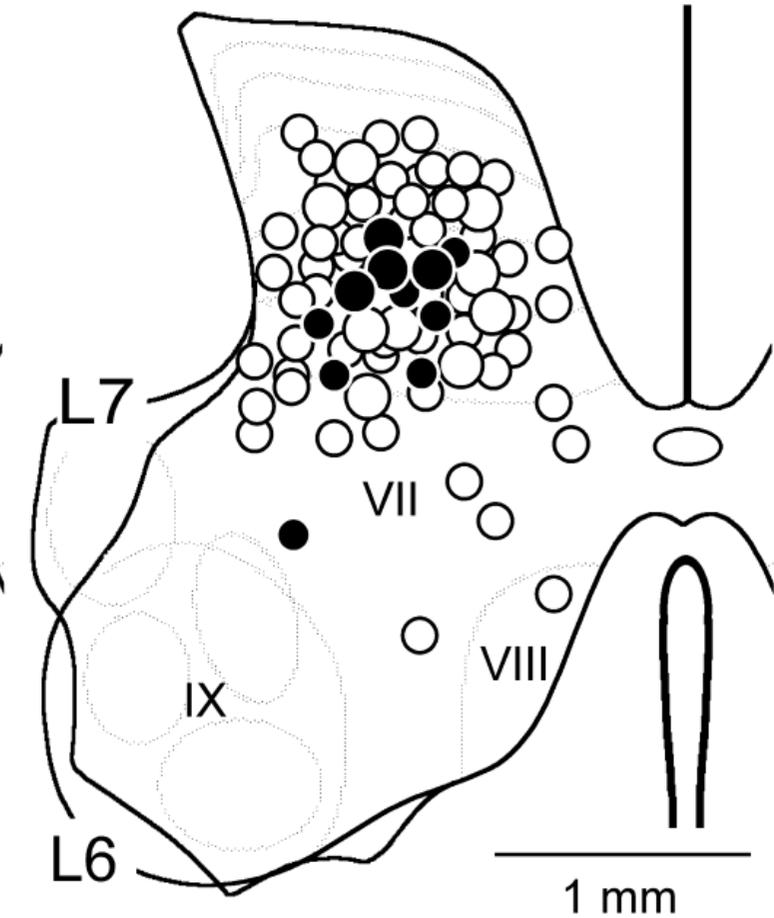




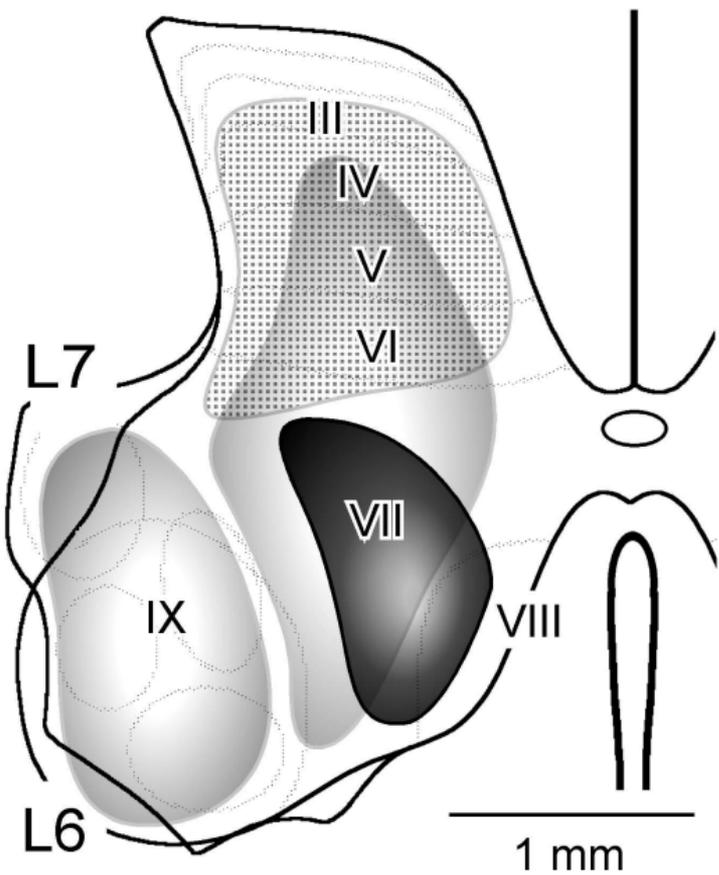
A a VR L7,S1**b** VRs L7,S1**C** NRGc 40 μ A**d** NRGc 40 μ A**e** NRGc**B** NRGc stim.



A**Actions from FRAs****Late NRGc effects****EPSPs**
(n=20)**No PSPs**
(n=10)**IPSPs**
(n=25)**B****Spontaneous firing frequency****Late NRGc responses****Excitation**
(n=35)**Unchanged**
(n=59)**Inhibition**
(n=42)

A Mono. excitation**B** Late excitation**C** Late inhibition**D** Lack of late responses

A Spinal cord organization



NRGc effects

Interneurons

● Excitation

● Inhibition

● unaffected

Motoneurons

● Inhibition

B Proposed interneurons

