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Takakusaki K, Habaguchi T, Ohtinata-Sugimoto J, Saitoh K, and Sakamoto T.

Department of Physiology II, College of Medicine, Asahikawa Medical College,

Midorigaoka-Higashi, 2-1, Asahikawa 078-8510, JAPAN.

Author for correspondence

Kaoru Takakusaki MD, PhD
Associate Professor
Department of Physiology
Asahikawa Medical College
Midorigaoka-Higashi, 2-1
Asahikawa 078-8510, JAPAN.
Tel: +81-166-68-2332
Fax: +81-166-68-2339
E-mail: kusaki@asahikawa-med.ac.jp (K. Takakusaki)

Abbreviations:

ChAT, choline acetyltransferase;
CNF, cuneiform nucleus;
CP, cerebral peduncle;
CTX, cerebral cortex;
CS, centralis superior;
EMG, electromyogram;
GABA, γ-amino butyric acid;
GPi, internal segment of globus pallidus;
IC, inferior colliculus;
MLR, mesencephalic locomotor region;
MRF, medullary reticular formation;
NMDA, N-methyl-D-aspartate;
NRPo, nucleus reticularis pontis oralis;
PAG, periaquaductal grey;
PPN, pedunculopontine tegmental nucleus;
PRF, pontine reticular formation;
RD, raphe dorsalis;
RN, red nucleus;
SC, superior colliculus;
SNc, substantial nigra pars compacta;
SNr, substantia nigra pars reticulata;
REM, rapid eye movement;
SCP, superior cerebellar peduncle;
SO, superior olive;
Sol, soleus;
TB, triceps brachii;
V, trigeminal nucleus.

Abstract

The present study is designed to elucidate how basal ganglia afferents from the substantia nigra pars reticulata (SNr) to the mesopontine tegmental area of the brainstem contribute to gait control and muscle tone regulation. We used unanesthetized and acutely decerebrated cats (n=27) in which the striatum, thalamus and cerebral cortex were removed but the SNr was preserved. Repetitive stimulation (50 Hz, 10–60 μA, for 5–20 s) applied to a mesencephalic locomotor region (MLR), which corresponded to the cuneiform nucleus, and adjacent areas, evoked locomotor movements. On the other hand, stimulation of a muscle tone inhibitory region in the pedunculopontine tegmental nucleus (PPN) suppressed postural muscle tone. An injection of either glutamatergic agonists (N-methyl-D-aspartic acid and kainic acid) or γ-amino butyric acid (GABA) antagonists (bicuculline and picrotoxin) into the MLR and PPN also induced locomotion and muscle tone suppression, respectively. Repetitive electrical stimuli (50–100 Hz, 20–60 μA for 5–20 s.) delivered to the SNr alone did not alter muscular activity. However stimulating the lateral part of the SNr attenuated and blocked PPN-induced muscle tone suppression. Moreover, weaker stimulation of the medial part of the SNr reduced the number of step cycles and disturbed the rhythmic alternation of limb movements of MLR-induced locomotion. The onset of locomotion was delayed as the stimulus intensity was increased. At a higher strength SNr stimulation abolished the locomotion. An injection of bicuculline into either the PPN or the MLR diminished the SNr effects noted above.

These results suggest that locomotion and postural muscle tone are subject to modulation by GABAergic nigrotegmental projections which have a partial functional topography: a lateral and medial SNr, for regulation of postural muscle tone and locomotion, respectively. We conclude that disorders of the basal ganglia may include dysfunction of the nigrotegmental (basal ganglia-brainstem) systems, which consequently leads to the production of abnormal muscle tone and gait disturbance.

Key words

Substantia nigra pars reticulata

GABAergic projection

Mesencephalic locomotor region

Pedunculopontine tegmental nucleus

Parkinson’s disease

Decerebrate preparation

Running title: Basal ganglia control of postural muscle tone and locomotion

The current understanding is that the basal ganglia and cerebellar loops with the motor areas of the cerebral cortex are involved in the control of voluntary movements (Middleton and Strick, 2000a). Specifically, basal ganglia disorders are manifested by an inability to initiate voluntary movements, an inability to suppress involuntary movements, an abnormality in the velocity and amount of movement, and an abnormal muscle tone. Gait disturbances are also a major impediment for Parkinsonian patients (Murray et al., 1978; Morris et al., 1994). Additional evidence indicates that the basal ganglia contribute to the planning and execution of voluntary movements via a series of parallel basal ganglia thalamocortical loops (Delong, 1990; Middletone and Strick 2000b). But how the basal ganglia control muscle tone and gait performance is unclear. The basal ganglia outflow also reaches the brainstem (Inglis and Winn, 1995; Hikosaka et al., 2000) where fundamental neuronal networks for controlling muscle tone and locomotor movements (Grillner et al., 1981; Mori, 1987; Rossignol, 1996) are located. The lateral part of the mesopontine tegmentum includes the mesencephalic locomotor region (MLR) (Shik et a1. 1966; Garcia-Rill, 1991; Grillner, 1981; Rossignol, 1996) and the muscle tone inhibitory region in the pedunculopontine tegmental nucleus (PPN) (Kelland and Asdourian, 1989; Lai and Siegel, 1990; Takakusaki et al., 1997a). In rats (Beckstead et al., 1979; Spann and Grofova, 1991) and cats (Moriizumi et al., 1988) the mesopontine tegmentum receives efferents of the basal ganglia particularly from the substantia nigra pars reticulata (SNr). It is presumed that the nigrotegmental efferents use GABA as a neurotransmitter and have terminals preferentially on non-cholinergic neurons rather than cholinergic neurons (Grofova and Zhou, 1998; Rye et al., 1987).

In both decerebrate (Shik et a1., 1966; Garcia-Rill, 1991; Grillner, 1981) and alert (Mori et al., 1989) cats, locomotion has been evoked by repetitive electrical stimulation delivered to the MLR. It has been suggested by many studies that the MLR largely corresponds to the cuneiform nucleus (CNF) and a region of the PPN, and that non-cholinergic neurons in these areas are involved in the generation of locomotion (see Grillner et. al., 1997; Inglis and Winn, 1995; Jordan, 1998). Signals from the MLR activate

central pattern generators in the spinal cord mainly through the medullary reticulospinal tract (Garcia-Rill, 1991; Grillner et al., 1981; Rossignol, 1996). In addition, Garcia-Rill et al. (1985, 1990) reported that injecting GABA antagonists into the MLR evoked locomotion. They suggested that the locomotion was regulated by a sustained GABAergic influence from the SNr of the basal ganglia. In contrast, in decerebrate cats, repetitive electrical stimulation of the lateral part of the PPN suppressed muscle tone (Lai and Siegel, 1990; Takakusaki et al., 1997a). The PPN has a descending projection to the pontomedullary reticular formation, in addition to ascending projections to the basal ganglia and non-specific thalamic nuclei (Jackson and Crossman, 1983; Jones, 1991; Lai et. al., 1993; Mitani et al., 1988; Moon-Edley and Graybiel, 1983; Saper and Lowey, 1982; Semba and Fibiger, 1992; Shiromani et al., 1990; Takakusaki et al., 1996). It is possible therefore that the descending projection could be responsible for the motor inhibition via the pontomedullary reticulospinal tract (Chase and Morales, 1990; Takakusaki et al. 1994). However, how GABAergic basal ganglia efferents could modulate postural muscle tone via the PPN has not yet been substantiated.

A better understanding of the role of the basal ganglia in the control of postural muscle tone and locomotion may be possible by establishing how efferents of the basal ganglia modulate the activity of neuronal systems in the brainstem and spinal cord that control these functions. Consequently, we examined the effects of activation of the nigral GABAergic efferents on PPN/MLR-induced muscle tone suppression and locomotion. The experiments were performed using acute decerebrate cats in which the striatum, thalamus and cerebral cortex were removed, but the SNr was preserved. Because of the complexity of the structure of the mesopontine tegmentum, a chemical stimulation (an infusion of neuroactive substances) technique was utilized in addition to electrical stimulation so that the neuronal activity could be selectively altered. In the first part of the investigation, we re-examined the exact location of the inhibitory region in the PPN with reference to that in the MLR. We then investigated whether postural muscle tone was modulated by GABAergic afferents to the PPN and whether cholinergic PPN neurons contributed to

suppression of the muscle tone. In the latter, we examined how electrical stimulation of the SNr modulated the PPN-induced muscle tone suppression and the MLR-induced locomotion. The effective sites in the SNr for modulating these movements were also explored to determine if the SNr had a functional topography. Finally, we discuss the functional role of the basal ganglia-brainstem systems in the integrative process of postural muscle tone and locomotion, and propose a new concept for understanding the motor disturbances of basal ganglia disorders. The preliminary results of the present investigations have been published elsewhere as abstracts (Habaguchi et al. 1998; Takakusaki et al. 1997a, 2000).

Experimental procedures

The experiments were performed with laboratory-raised cats (n=27) weighing from 2.2 to 3.8 kg. All of the procedures which were used in this study were approved in the Guide for the Care and Use of Laboratory Animals (NIH Guide), revised 1996. During the investigation every effort was made to minimize animal suffering and to reduce the number of animals used.

Surgical procedures

We employed two types of decerebrate preparations. One type of preparation was decerebrated at the precollicular-postmammillary level and the other was decerebrated at the precollicular-premammillary level (see Drew and Rossignol 1984). Each animal was anesthetized with halothane (Halothane, Hoechst: 0.5-3.0%) and nitrous oxide gas (0.5-1.0 l/min) with oxygen (3.0-5.0 l/min), the trachea was intubated, and a decerebration was surgically performed. A catheter was placed in the femoral artery to monitor the blood pressure. The anesthesia was then discontinued. The animal's head was fixed in a stereotaxic apparatus and a rigid spinal frame securely held the cat by clamping the dorsal processes of the first 3 thoracic vertebrae. The limbs rested on a static surface or the belt of a treadmill while the body was supported by a rubber hammock. The animal's rectal temperature was maintained at 36 to 37°C by using radiant heat lamps. Each cat's mean blood pressure was maintained at greater than 100 mmHg, and the end tidal CO_2 was maintained between 4 and 6%.

Brainstem stimulation and EMG recording

A glass micropipette filled with Wood's metal, and with the tip replaced by a carbon fiber (diameter 7 μm, resistance 0.2 to 0.5 MΩ; Takakusaki et al. 1994, 2001) was inserted into the mesopontine tegmentum (A 1.0 to P 3.0, LR 2.0 to 5.0, H +1.0 to –5.0). When the animal's limbs rested on a stationary surface, repetitive stimuli with a constant pulse (10 to 60 μA and 0.2 ms duration at 10 to 200 Hz) were delivered for 5 to 30 s to the

mesopontine tegmentum to inhibit any muscular tonus. Locomotion was evoked by repetitive stimuli (10 to 60 μA and 0.2 ms duration at 50 Hz) which were delivered while the treadmill belt was moving at a speed of 0.3 m/s. To identify the optimal site for evoking muscle tone suppression and locomotion, the stimuli were delivered at 0.5 to 1.0 mm intervals in the dorsoventral, mediolateral and rostrocaudal directions (Figs.1 and 2). An identical type of electrode was also inserted into the caudal diencephalon (A 2.0 to 5.0, LR 3.0 to 7.0, H +2.0 to –5.0) to stimulate the SNr with a constant pulse (10 to 60 μA for 0.2 ms duration at 10 to 200 Hz). The stimuli were delivered at 0.5 to 1.0 mm intervals in the dorsoventral, mediolateral, and rostrocaudal directions so that the optimal stimulus sites for inhibiting the MLR and PPN-induced effects could be identified (Fig.12).

A micropipette which was filled with one of the following neuroactive substances was inserted into the mesopontine tegmentum (P 0 to 2.5, LR 3.5 to 4.5, H –1.0 to –3.5): γ-amino butyric acid (GABA; 2.0 to 10 mM); muscimol (2.0 to 10 mM); picrotoxin (1.0 to 5.0 mM); bicuculline (1.0 to 5.0 mM); kainic acid (0.1 to 0.5 mM); and N-methyl-D-aspartate (NMDA; 0.1 to 0.5 mM). By using an oil-driven microinjection system, each substance was injected into the target area where the electrical stimulation had produced locomotion or muscle tone suppression. The volume of each injection was invariably 0.2 to 0.25 μl and the injection rate was 0.01 to 0.02 μl/s. In addition, a micropipette filled with atropine sulfate (10 to 20 mM) was vertically inserted into the medial pontine reticular formation (PRF; P 2.0 to 3.0, LR 1.5 to 2.5, H –3.0 to –4.0) where repetitive electrical stimulation had abolished muscular tonus (Fig.1 Be; Oka et al. 1993, Takakusaki et al. 1994). The volume of atropine which was injected was from 0.2 to 0.5 μl and the injection rate was from 0.01 to 0.02 μl/s. All of the substances were dissolved in Ringer solution with the pH adjusted to 7.4. A pair of stainless steel wires 2 mm apart were inserted into the bilateral triceps brachii (TB) and soleus (Sol) muscles to record the electromyographic (EMG) activity from each muscle.

Histological verification

At the end of an experiment the stimulus sites were marked by passing a DC current of 30 μA through an electrode for 30 s. The injection sites and any spreading of the infusates were marked by an infusion of an equal volume of 10% Fast Green. Each cat was sacrificed with an overdose of Nembutal anesthesia and the brainstem was then removed. The brainstem was fixed in 10% formalin and later the frozen 50 μm coronal or parasagittal sections which were cut were stained with neutral red. The location of the microlesions and diffusion areas of the Fast Green were identified with assistance from the stereotaxic atlases of Berman (1968) and Snider and Niemer (1961), which were used as references.

ChAT immunohistochemistry

Four of the animals were deeply anaesthetized with Nembutal and transcardially perfused with 0.9% saline followed by a solution of 3.0% paraformaldehyde (0.01%) glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The brain of each animal was then removed, saturated with a cold solution of 30% sucrose, and 50 μm frozen sections were prepared. Choline acetyltransferase (ChAT) immunohistochemistry was then performed by using the peroxidase-antiperoxidase method combined with diaminobenzidine (Lai et al., 1993; Mitani et al., 1988). Monoclonal anti-ChAT antibody (Boehringer Mannheim) was used for these preparations.

Results

Topographical arrangement of the muscle tone inhibitory region and locomotor region

We first confirmed the optimal stimulus sites for evoking locomotion and muscle tone suppression. Repetitive stimuli with parameters of 30 μA and 50 Hz which were delivered to the ventral margin of the cuneiform nucleus (CNF; Fig.1A) evoked an alternating hindlimb loading. Locomotor movements developed when the treadmill started to move (a downward arrow in Fig.1Ba). Stimulation which was 1.0 mm ventral to the locomotion-evoking site elicited a tegmental reflex, i.e., flexion of the ipsilateral (right) hindlimb and extension of the contralateral (left) hindlimb (Fig.1Bb). When stimuli were delivered to the dorsal part of the PPN stepping movements of the hindlimb were evoked and these were subsequently attenuated along with a decrease in muscle tone (Fig.1Bc). On the other hand, stimulating the ventral part of the PPN immediately suppressed muscle tone (Fig.1Bd). The muscular atonia persisted, even after termination of the stimulation, until a pinna was stimulated by manually pinching the scapha (open triangle in Fig.1Bd). This muscular atonia resembled that induced by stimulating the medial PRF corresponding to the nucleus reticularis pontis oralis (NRPo; Fig.1Be; Oka et al. 1993, Takakusaki et al. 1994). In this animal the sites which evoked locomotion were located in the CNF, whereas the inhibitory sites were located in the ventrolateral part of the PPN and the NRPo (Fig.1C). The latency of the muscular suppression was shorter when either the stimulus intensity was increased (Fig.1Da–c) or the stimulus frequency was increased up to 100 Hz (Fig.1Db, d). However, stimuli with a frequency of 200 Hz did not abolish the muscle tone (Fig.1De).

The effects of PPN stimulation upon locomotor movements were examined in the precollicular-premammillary decerebrate cat (n=3). Shown in Fig.2 are the results from one cat which spontaneously walked on the treadmill with a cycle time of 0.7 s. Repetitive stimulation of the CNF changed the locomotor pattern from fast walking to a gallop (Fig.2Ba). The stimuli to the dorsal PPN initially facilitated but subsequently attenuated the locomotion (Fig.2Bb). In contrast, stimulating the ventral PPN immediately stopped the locomotion (Fig.2Bc). When the ventral PPN was stimulated with a lower strength, the

cycle time of the locomotion was gradually increased, the muscle tone was decreased, and the rhythmical movements of bilateral hindlimbs were diminished (Fig.2Bd). In this animal locomotion was suppressed (filled circles) by stimuli to the PPN, and facilitated (open circles) by stimuli to the CNF and the area rostral to this nucleus (Fig.2C).

The optimal sites for muscular atonia and locomotion in 14 animals are plotted on coronal (upper) and parasagittal (lower) planes of the brainstem in Fig.3A. The inhibitory sites were distributed in the ventrolateral part of the PPN (left panels). The distribution of the locomotor evoking sites is shown in the panels on the right side. The sites were located mainly in the CNF, and an adjacent area rostral and ventral to this nucleus, including the dorsal part of the PPN. In 7 animals a mixture of locomotion and muscular atonia was induced by stimuli between the two regions (middle panels). These findings suggest that in the mesopontine tegmentum there is a functional topography with respect to the control of locomotion and muscle tone. The distribution of cholinergic neurons labeled by ChAT immunohistochemistry is shown in Fig.3B. The cholinergic neurons were preferentially distributed in the area corresponding to the inhibitory region rather than the locomotor region. This observation indicated that the suppression of the muscle tone could possibly be ascribed to activation of cholinergic neurons.

On the basis of the above findings we have proposed a framework for the control of locomotion and muscle tone, which is schematically illustrated in Fig.4. It has been noted that cholinergic PPN neurons projecting to the PRF could possibly be involved in the suppression of muscle tone (Lai and Siegel, 1993; Mitani et al., 1988). This suppression could occur via the inhibitory reticulospinal tract that provides a postsynaptic inhibition of motoneurons, directly, or via an inhibitory spinal interneuron (inhibitory system; Chase and Morales, 1990; Takakusaki et al., 1994). Furthermore, signals from the MLR possibly activate central pattern generators in the spinal cord mainly through the medullary reticulospinal tract (locomotor system; Grillner et al., 1981; Rossignol, 1996). We postulated that the GABAergic basal ganglia efferents arising from the SNr to the MLR

and the PPN control locomotion and muscle tone, respectively. The following experiments were performed on the basis of this framework.

Injections of putative transmitters into the brainstem modulate muscle tone and locomotion

To test whether locomotion and muscle tone suppression could be induced by an activation of neurons located in the MLR and PPN, we studied the effects of injecting glutamatergic and GABAergic agents into the mesopontine tegmentum (Fig.5). An injection of NMDA into the left CNF bilaterally increased the hindlimb muscle tone (Fig.5Ab) and locomotor movements were elicited when the treadmill started to move (indicated by a downward arrow in Fig.5Ac). In contrast, an NMDA injection into the left PPN, 2 hours after the first injection, abolished the muscle tone (Fig.5Ad). The muscular atonia had continued for about 6 min until the left pinna was pinched. In the same animal, an injection of kainic acid into the right PPN also abolished muscle tone (Fig.5Ae). An injection of glutamatergic agonists into the PPN in the other 3 animals invariably suppressed muscle tone. Muscimol (2 cats) and GABA (2 cats) were also injected into the CNF where the repetitive stimulation induced locomotion. These injections attenuated and finally blocked the electrically-induced locomotion (not illustrated). These findings are essentially the same as those demonstrated by Garcia-Rill et al. (1985).

We also inquired as to whether postural muscle tone was modulated by GABAergic afferents to the PPN (Fig.5Ba). An injection of muscimol into the PPN did not affect the level of muscle tone (Fig.5Bb), but it obviously reduced the PPN effect. Stimulation of the PPN, which had abolished muscle tone before muscimol (Fig.5Bc), now did not suppress it (Fig.5Bd). To reduce the muscle tone PPN stimulation at a higher intensity (60 μA) and for a longer duration (20 s) was required (Fig.5Be). The effects of muscimol and GABA were examined in the other 4 cats, and essentially the same findings were obtained.

Then what happened if the GABAergic afferents to the MLR and the PPN were removed? To remove GABAergic inputs, $GABA_A$ antagonists were injected into the mesopontine tegmentum (Fig.6). Injecting bicuculline into the area rostral to the CNF (“b”

in Fig.6Ad) increased the level of muscle tone and elicited quadrupedal locomotion with a cycle time of 0.7 cycles/s (Fig.6Ab). But a subsequent bicuculline injection into the ventral PPN ("c" in Fig.6Ad), which was performed at about 20 min after the first injection, gradually suppressed the locomotion and produced an associated decrease in muscle tone (Fig.6Ac). The results illustrated in Fig.6B, which are from a different cat, display the effects of an injection of picrotoxin, another $GABA_A$ antagonist, into the ventral PPN. The injection suppressed the muscle tone of both forelimbs and hindlimbs (Fig.6Ba). During the muscular atonia the pinna was pinched at approximately 2 min and 20 min (indicated by triangles) but the level of muscle tone was not re-established. The muscular atonia lasted until the muscle tone was restored by pinching the pinna at approximately 80 min. An example of a bicuculline injection that evoked a mixture of muscular atonia and locomotion is shown in Fig.6C. An injection of bicuculline into the lateral part of the PPN initially suppressed muscle tone. Subsequently, restoration of the level of muscle tone commenced (Fig.6Ca) and this restoration of muscle tone was associated with the development of stepping movements (shown in the records with an expanded time scale in Fig.6Cb). These stepping movements ceased when the muscle tone reached a higher level. When the muscle tone started to decrease the stepping movements were recommenced, but the rhythmical movements were diminished together with the reduced muscle tone (Fig.6Cc). The sequence of the alternation of muscular atonia and stepping movements persisted for approximately 60 min.

In all, GABA antagonists were injected into 24 sites in 18 animals (Fig.7). Fast green was used to identify the injection sites and to a measure the spread of the infusions which for each injection was limited to an area of approximately 1.0 to 1.5 mm in diameter. Nine of the injections induced a muscular atonia with latencies from 24 s to 7 min (Fig.7A). These injection sites were concentrated in the area corresponding to the ventrolateral part of the PPN. On the other hand, locomotion was induced (Fig.7C) within 10 min by injection into the medial part of the CNF in 2 cats, and with latencies from 14 to 26 min by another 4 injections into the area rostroventral to the CNF. A mixture of muscular atonia

and locomotion was observed in 3 trials in which the injection sites were located between the inhibitory region and the locomotor region (Fig.7B).

Because the cholinergic neurons were dense in the area corresponding to the inhibitory region in the PPN, we investigated whether the cholinergic PPN neurons projecting to the PRF could be involved in the suppression of muscle tone (Fig.8). The effects of an injection of atropine sulfate, a muscarinic antagonist, into the medial PRF on electrically (Fig.8Aa) and chemically (Fig.8Ba) -induced muscular atonia were tested. Although PPN stimulation suppressed muscle tone (Fig.8Ab), the same stimulation did not after an atropine injection into the medial PRF (Fig.8Ac). On the other hand, the atropine injection facilitated the MLR-induced locomotion (Fig.8Ae and Af). After approximately 40 min the PPN and MLR effects recovered (Fig.7Ad and Ag). An atropine injection also blocked muscular atonia that was induced by a bicuculline injection into the PPN (Fig.8B). During the atonia, the muscle tone did not recover even when the bilateral triceps surae muscles were manually stretched (closed triangle in Fig.8Bb). However, when stretching the triceps surae muscles was combined with an atropine injection into the left PRF (approximately 37 min after the bicuculline application), muscle tone was partially restored. An additional atropine injection into the right PRF and stretching of the hindlimb muscles finally re-established tonic muscle contractions (Fig.8Bc). Eventually a bicuculline injection into not only the left (Fig.8Bc) but also the right (not illustrated) PPN failed to reduce the muscle tone.

GABAergic nigrotegmental projections control postural muscle tone and locomotion

As a final step of this study, we elucidated how the GABAergic projections from the SNr to the mesopontine tegmentum modulate postural muscle tone and locomotion (Figs. 9–11). Repetitive stimulation of the SNr (indicated by an open circle in Fig.9E) did not alter muscle tone (Fig.9Ab). However, stimulation of the SNr with a relatively lower current (30 to 40 μA) attenuated PPN-induced muscle tone suppression (Fig.9Ac–d). We also noted that stimuli with a higher current (50 μA) finally blocked the PPN-effect

(Fig.9Ae). The SNr stimulus effects depended on the stimulus frequency (Fig.9B). While SNr stimuli of 100 Hz completely blocked the PPN-effects, stimuli with either a lower (20 Hz) or higher (200 Hz) frequency did not. In this animal the electrode for stimulating the SNr was moved so that the optimal site for blocking the PPN-effects could be determined (Fig.9C and E). The most effective stimulus site was located in the dorsolateral part of the SNr. Stimulation sites which were either dorsal or ventral attenuated the SNr effects.

It is noteworthy that SNr stimulation modulated locomotor movements (Fig.10). Although several seconds of stimulation were usually required to elicit locomotion, locomotion in this animal was elicited immediately after the start of MLR stimulation (Fig.10Cb). Conditioning SNr stimuli with a lower current (10 to 20 μA) increased the cycle time, reduced the locomotor speed, and disturbed the rhythmic alternation of limb movements (Fig.10Da and b). When the stimulus intensity was increased (30 to 40 μA) the limb movements became slower and muscle activity was reduced in amplitude (Fig.10Dc and d). Moreover the onset of locomotion was delayed when the stimulus intensity was increased. The locomotor movements were eventually abolished at a stimulus intensity of 50 μA (Fig.10De). As shown in the graphs in Fig.10E, both the cycle time and the latency to the onset of locomotion were increased as the stimulus intensity was increased. These SNr-effects were prominent when the stimulus frequency was 50 to 100 Hz (Fig.10F). Stimuli with higher frequencies (143 to 200 Hz) were less effective.

To reveal whether the effects of the SNr which are noted above were mediated by a GABAergic projection, the SNr was stimulated after injecting bicuculline into the PPN (4 cats) and MLR (3 cats). Before a bicuculline injection SNr stimulation restored the muscle tone that was suppressed by PPN stimulation (Fig.11Ab). However the SNr stimulation failed to alter the muscle tone that was suppressed by a bicuculline injection into the PPN (Fig.11Ac). Similarly, SNr stimulation, after either injecting bicuculline (2 cats) or picrotoxin (1 cat) into the CNF, did not block MLR-induced locomotion (Fig.11Bc).

The effective sites in the SNr for blocking the PPN/MLR-effects are shown in Fig.12. On the coronal plane (Fig.12Aa) the PPN-effects were effectively blocked when

stimuli were delivered to the dorsolateral part of the SNr and to the adjacent mesencephalic reticular formation dorsal to the SNr. On the parasagittal plane the effective sites were rostrocaudally extended at the dorsal part of the SNr (Fig.12Ab). In contrast, the effective sites for inhibiting locomotion were found in the middle and medial parts of the SNr (Fig.12Ba), and these were rostrocaudally distributed for the whole length of the SNr (Fig.12Bb).

All the above results suggest that both “the muscle tone inhibitory system” and “the locomotor system” are under the control of sustained GABAergic inhibition from the SNr. Moreover, the GABAergic nigrotegmental projections can be functionally organized; i.e., the projections from the lateral and medial SNr may regulate muscle tone and locomotion, respectively (Fig.13A).

Discussion

In the present study we provide new evidence that the basal ganglia efferents to the brainstem centers controlling postural muscle tone and locomotion. Before consideration of the functional role of the basal ganglia-brainstem system, the experimental procedures are discussed in relation to the complexity of the structure of the mesopontine tegmentum. Finally, we propose a new concept of the mechanisms by which the basal ganglia control movements and discuss this with respect to motor disturbances in basal ganglia disorders.

Consideration of the experimental procedures

The stimulus strength in this study was less than 60 μA and the current spread was estimated to be under 0.5 mm because displacement of the stimulating electrode by 0.5 mm evoked different effects. Even so, the electrical stimuli activated both neuronal elements and fibers of passage, because numerous ascending and descending fibers are intermingled with cholinergic (Armstrong et al. 1983; Rye et al. 1987; Span and Grofova 1992) and non-cholinergic neurons. The latter include glutamatergic (Clements et al. 1991), GABAergic (Ottersen and Storm-Mathisen 1984; Krosaka et al. 1988), and peptidergic (e.g., Substance P; Vincent et al. 1983) neurons in the mesopontine tegmentum.

Chemical stimulation is suitable for activation of neuronal elements and for supplementing any electrical stimulation. The effects of drug injection, however, depend on factors such as the receptor density of cells at the injection site, the diffusion delay, and the time required for the recruitment of neurons related to each movement (Takakusaki et al. 1993; Yamamote et al. 1990). Consequently the effects of either electrical or chemical stimulation are difficult to ascribe to the activation of a particular neurotransmitter system. Indeed, either type of stimulus often evoked a mixture of muscular atonia and locomotion. Therefore the neurons responsible for each movement were possibly co-localized at the site where either type of stimulus was delivered. Alternatively the stimulus current or drug infusion might spread to both regions and evoke the mixed effects.

Neuronal substrates in the mesopontine tegmentum involved in the control of locomotion and postural muscle tone

The MLR has been established as a functional region involved in the initiation of locomotion on the basis of its connections with limbic structures and the basal ganglia (Armstrong, 1986; Mogenson et al., 1993). The CNF and the PPN have been considered as the major components of the MLR (Grillner et al., 1997). Based on the following findings, Garcia-Rill (1991) convincingly suggested that an activation of cholinergic neurons in the PPN is required to initiate locomotion. First, locomotion was induced by electrical or chemical stimulation within the PPN pars compacta where cholinergic neurons were abundantly located (Garcia-Rill et al., 1987). Second, MLR-induced locomotion was blocked by injections of atropine sulfate into the medioventral medulla where efferent fibers from the PPN terminate (Garcia-Rill and Skinner, 1987). Because injections of GABA antagonists and NMDA into the PPN elicited locomotion, they further suggested that the cholinergic neurons receive both GABAergic and glutamatergic inputs from forebrain structures (Garcia-Rill et al., 1985, 1990).

The present results, however, are not consistent with their observations on the following two points. First, the locomotor region was mainly located within, and around the CNF, including the dorsal part of the PPN. This location corresponds well to that noted in most of the studies reviewed by Grillner et al. (1997). Locomotion also was facilitated by stimuli applied to area rostral to the CNF (Fig.2). Electrical stimuli possibly activated either fibers from the subthalamic nucleus (Kita and Kitai, 1987) and/or subthalamic locomotor region projecting to the MLR (Rossignol, 1996) or dendrites of CNF neurons. Second, cholinergic neurons were mostly distributed in the inhibitory region rather than the locomotor region. We consider that non-cholinergic neurons are the major components of the MLR. Previous findings support this idea. An expression of c-fos following treadmill locomotion in rats has been detected in the CNF and not in the PPN (Shojania et al., 1992). Similarly, labeling with 2-deoxyglucose in cats has revealed an increased activity only in the CNF as result of MLR-induced locomotion (Shimamura et al., 1987).

Our results suggest that cholinergic neurons within the PPN contribute to the control of muscle tone. An inhibitory region was located in the ventrolateral part of the PPN, and the location coincides with that reported by Lai and Siegel (1990). We further observed that $GABA_A$ receptors, in addition to non-NMDA and NMDA glutamate receptors of the cholinergic neurons, were involved in this process. The cholinergic neurons possibly link with medial PRF neurons, which subsequently excite the muscle tone inhibitory system (Chase and Morales, 1990; Takakusaki et al., 1994). Lai and Siegel (1988, 1991) have also reported that injection of non-NMDA agonists into the medial PRF and ventromedial medullary reticular formation (MRF) inhibited muscle tone. Taken together, both the cholinergic and glutamatergic PPN neurons projecting to the PRF (Lai and Siegel 1993; Mitani et al., 1988) and MRF (Shiromani et al. 1990) may regulate muscle tone.

For the following two reasons, we cannot disregard the contribution of cholinergic PPN neurons to the control of locomotion. First, an atropine injection into the PRF increased step cycles of MLR-induced locomotion associated with an enhancement of muscle tone (Fig.8). Second, injecting GABA antagonists into the PPN initiated and terminated locomotor movements, depending on the level of muscle tone (Fig.6C). We emphasize that cholinergic PPN neurons can be involved in both the initiation and termination of locomotion and the maintenance of the locomotor movements through their capability of modulating postural muscle tone. Garcia-Rill and Skinner (1988) observed in a fictive locomotion preparation that neurons within the PPN displayed a tonic firing property which was preferentially related to the onset and/or termination of locomotion. They observed however, that neurons in the CNF discharged preferentially in a bursting pattern that was linked to the locomotor cycles. Their findings, together with the present results, suggest that there is a functional topographical organization in the mesopontine tegmentum. Moreover, if the two types of neurons are cholinergic and non-cholinergic respectively, their interaction within the mesopontine tegmentum could play an important role in the integration of postural muscle tone and locomotion.

GABAergic basal ganglia inputs to the mesopontine tegmentum

In the mesopontine tegmentum of rats (Beckstead et al., 1979; Span and Grofova, 1991) and cats (Moriizumi et al. 1988) the most dense basal ganglia efferents are from the SNr. The mesopontine tegmentum in primates also receives efferents from the internal segment of the globus pallidus (GPi; Nauta and Mehler 1966). In non-primates the same area is referred to as the entopeduncular nucleus (Moriizumi and Hattori, 1992). In addition, there are efferents from the nucleus accumbens (Groenewegen et al., 1993) via the ventral pallidum, including the substantia innominata (Swanson et al., 1984). We cannot, therefore, disregard the possibility of a contribution of these pallidotegmental fibers to the nigral stimulus effects. It is also presumable that the SNr regulates locomotion and muscle tone by modulating the activity of the medullary reticulospinal tract via a direct nigrobulbar projection (Schneider et al. 1985). However, this may be unlikely because the SNr effects were usually blocked by injecting GABA antagonists into the mesopontine tegmentum.

It is worth noting that there is a functionally organization in the nigrotegmental projections; i.e., the lateral and medial part of the SNr project to the PPN and the MLR, respectively (Fig.13A). A similar organization of the nigrotegmental projections has been demonstrated in a neuroanatomical study (Moriizumi et al., 1988). This study revealed that the medial PPN, which approximately corresponds to the MLR, receives afferents from the medial half of the SNr, whereas the lateral PPN, which corresponds to the inhibitory region, receives afferents from the lateral half of the SNr. Most neuroanatomical studies have emphasized that the primary targets of the nigrotegmental fibers are non-cholinergic PPN neurons (Beckstead et al., 1979; Grofova and Zhou, 1998; Moriizumi et al., 1988; Rye et al., 1987; Spann and Grofova, 1991). Only a few studies have suggested a nigrotegmental projection to cholinergic PPN neurons (Grofova and Zhou, 1998; Semba and Fibiger, 1992). Because electrical stimulation of the SNr evoked monosynaptic IPSPs in cholinergic PPN neurons (Kang and Kitai, 1990; Takakusaki et al. 1997b), we prefer the idea that the nigrotegmental projection to the cholinergic PPN neurons is responsible for muscle tone regulation even though this projection is small. The nigrotegmental projection to the

non-cholinergic neurons may be rather involved in the control of locomotion as suggested by Inglis and Winn (1995).

Basal ganglia control of postural muscle tone and locomotion

The present results suggest that cholinergic PPN neurons activate muscle tone inhibitory system. Monoaminergic systems such as the coerulospinal (Fung and Barnes, 1981) and raphespinal (Sakai et al. 2000) tracts are considered as muscle tone facilitatory systems. There are also serotonergic projections to the PPN (Honda and Semba, 1994) and to the medial PRF (Semba and Fibiger, 1992). It seems that the former inhibits midbrain cholinergic neurons (Leonald and Llinas, 1994), and the latter reduces the activity of the inhibitory system (Takakusaki et al. 1994). In contrast, the inhibitory system suppresses the activity of the coerulospinal tract (Mileykovskiy et al., 2000). Thus muscle tone can be regulated by a counterbalance between the inhibitory and the facilitatory systems. An activation of the MLR is likely to excite the facilitatory systems (Mori et al. 1987). Indeed, injections of either NMDA or bicuculline into the MLR increased muscle tone (Figs.5 and 6). Nevertheless, SNr stimulation alone did not alter the level of muscle tone, although it greatly attenuated the PPN/MLR-effects (Figs. 9 and 10). Therefore the nigral efferents may regulate the activity of the inhibitory system to a larger extent than the facilitatory systems. We postulate that the basal ganglia-brainstem system regulate muscle tone by preferentially modulating the activity of the inhibitory system, which subsequently alters the inhibitory drive acting on the facilitatory systems.

Stimulation of the SNr increased the cycle time, disturbed the rhythmic limb movements and delayed gait initiation. These results indicate that the nigrotegmental projection affects both the steady state (e.g., postural control and rhythmic limb movements) and dynamic state (e.g., initiation and termination) of locomotion. It is considered that a deficiency of gait initiation (gait dyskinesia) in Parkinsonian patients can be ascribed to an inability of motor planning and programming at the level of the premotor and supplementary areas of cortex (Hanakawa et al. 1999; Murray et al., 1978; Morris et

al., 1994; Pahapill and Lazano, 2000). Because the SNr-induced changes in the locomotor patterns resemble the gait deficiencies of Parkinsonian patients, it is natural to consider that the basal ganglia control locomotor movements via projections to the brainstem in addition to its loops with the cerebral cortex.

The effects of SNr stimulation on muscle tone and locomotion were prominent if stimuli with frequencies of 50 to 100 Hz were used. These frequencies are almost the same as the spontaneous firing rate of SNr neurons in the alert monkey (Hikosaka and Wurtz, 1985). A frequency within this range can be a critical determinant in the control of muscle tone, locomotion, and saccadic eye movements (Hikosaka et al., 2000). However, the SNr effects were attenuated when high-frequency stimulation (150 to 200Hz) was applied. This phenomenon leads to an important concept: an intervention which blocks the abnormal activity of the SNr in Parkinsonism by high frequency stimulation could potentially restore the normal function of the brainstem centers that control muscle tone and locomotion. Similarly, the effects from the PPN were reduced when it was stimulated at a high frequency (200 Hz). Various mechanisms, including depolarization block and stimulation-evoked release of GABA caused by the high-frequency stimulation, have been proposed for the inactivation of neurons in the basal ganglia nuclei (Dostrovsky and Lozano, 2002; Vitek, 2002). These mechanisms could be acting on neurons in the SNr and the PPN.

An integration of “the locomotor system” and “the muscle tone control system” is essential to elicit a variety of locomotor pattern (Mori, 1987). The mesopontine tegmentum receives afferents from the cerebral cortex, the limbic systems, and hypothalamus, in addition to the basal ganglia (Armstrong, 1986; Grillner et al., 1997; Matsumura et al., 2000; Mogenson et al., 1993). The projections which relate to motor control and emotional control from these forebrain structures may contribute to this integrative process so that an animal can elicit a variety of locomotor behavior, depending on the behavioral context.

Basal ganglia control of movements; a new concept for understanding pathophysiological mechanisms of basal ganglia motor disorders

The majority of motor cortical neurons significantly increase their discharge rate when a walking subject has to overcome obstacles accurately (Drew et al., 1996). This accuracy requires a precise, visually-initiated, gait modification, in which the subject must modify their limb trajectory for each step, so that an appropriate foot placement can be achieved (Georgopoulos and Grillner, 1989). The basal ganglia and cerebellar loops with the cerebral cortex can assist such an accurate control, because these multiple loops contribute to both motor and non-motor functions including cognitive and sensory operations (Middleton and Strick, 2000b). On the other hand, the basal ganglia-brainstem system can be partly involved in automatic motor processes. The latter include the regulation of muscle tone and rhythmic limb alteration during locomotion. The motor cortices have projections to the PPN (Matsumura et al., 2000) and to the pontomedullary reticular formation (Matsuyama and Drew 1997). Therefore the muscle tone control system and the locomotor system can be controlled, in parallel, by a combined input to the brainstem of net inhibition from the basal ganglia, and net excitation from the motor cortex. A variety of locomotor patterns are then induced.

Given the above consideration, we have proposed a scheme for understanding the control of movement by the basal ganglia (Fig.13). There are multiple basal ganglia-thalamocortical loops with various areas of the cerebral cortex which are concerned with different aspects of motor behavior (Middleton and Strick, 2000a). In particular, the loops with the motor cortical areas are involved in the planning and execution of movements (Brooks, 1995). The motor cortical neurons that receive basal ganglia output may control the velocity and the amount of movement (abscissa on the left of the graph in Fig.13B; Delong et al., 1984; Turner and Anderson, 1997). Moreover the present results suggest that a GABAergic projection from the medial SNr to the MLR may control the locomotor pattern (abscissa on the right) and that from the lateral SNr to the PPN may determine the level of muscle tone (ordinate). Locomotion and muscle tone can be independently

controlled by the separate nigrotegmental projections. Because the basal ganglia output is variable in a normal condition, the degree of freedom for the amount of movement, the locomotor velocity, and the muscle tone, can be large. Each parameter can take any of the coordinates within the frame in Fig.13Ba.

This scheme may also assist understanding the mechanisms of motor disturbances in basal ganglia diseases. The GABAergic basal ganglia output is thought to be overactive in Parkinsonian patients (Delong, 1990; Witchmann and Delong, 1996). An excessive inhibition of thalamocortical neurons may reduce the velocity (bradykinesia) and the amount (hypokinesia) of movements. An increase in SNr inhibition, together with a decrease in cortical excitation of the PPN, may therefore increase the level of muscle tone (hypertonus). Similarly, an excessive inhibition of the MLR and a decrease in cortical excitation of the brainstem may elicit gait failure. As a result, the degree of freedom for each movement would be restricted, and the frame will be smaller and move to the upper right (Fig.13Ca). In contrast, a reduction of output from the basal ganglia in Huntington's disease may increase movement (hyperkinesias) and decrease muscle tone (hypotonus). The frame, which indicates the degree of freedom for movement, would be restricted and move to the lower right for this disease (Fig.13Cb). From these considerations, we hypothesize that the output of the basal ganglia would determine the degree of freedom of each movement, and a restriction of the degree of freedom could exist in the background of basal ganglia diseases.

Conclusion

Basal ganglia disorders are composed of a spectrum of abnormalities that range from hypokinetic-hypertonic syndromes at one extreme to hyperkinetic-hypotonic syndromes at the other. Both extremes of the spectrum can be explained by postulating a specific disturbance of basal ganglia output to both the motor cortex and brainstem. Here we have proposed, as a pathophysiological basis, that basal ganglia diseases may include

dysfunction of basal ganglia-brainstem systems which consequently produce dystonia and gait failure.

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Figure Legends

Figure 1. Mesencephalic stimulus sites evoking locomotion and muscle tone suppression. A. Stimulus sites in the mesopontine tegmentum on a coronal plane at P 2.0. B. Effects on muscle tone following the stimuli to each site in A. Each trace was obtained from the left (L) and right (R) soleus (Sol) muscles. A downward arrow in (a) indicates the onset of the treadmill with a speed of 0.3 m/s. An open triangle indicates stimulation applied to the left pinna by pinching the scapha. The stimulus parameters were: intensity, 40 μA; frequency, 50 Hz; duration, 5-10 s. C. Effective stimulus sites from which locomotion (open circles) and muscular atonia (filled circles) were evoked. Stimulation between the two regions evoked mixed effects (hatched square). Sites from which muscular atonia or locomotion were elicited by stimuli with intensities at 20 μA and 40 μA are indicated by large and small symbols, respectively. D. Effects of PPN stimulation following changes in the stimulus parameters. The stimulus intensity was increased from 20 μA (a) and 30 μA (b) to 50 μA (c). The stimulus frequency was increased from 50 Hz (b) and 100 Hz (d) to 200 Hz (e). The stimulus period is indicated by a line under each recording. An appropriate location of the PPN, which contains cholinergic neurons, is indicated by the grey colored area. SC, superior colliculus; IC, inferior colliculus; CNF, cuneiform nucleus; SCP, superior cerebellar peduncle; PPN, pedunculopontine tegmental nucleus; NRPo, nucleus reticularis pontis oralis.

Figure 2. Mesencephalic stimulus effects on spontaneous locomotion in the precollicular-premammillary decerebrate cat.

A. Stimulus sites on a parasagittal plane at L 4.5. B (a) – (c). Effects of repetitive stimuli (30 μA, 50 Hz, duration approximately 5 s) to each site in A on spontaneous locomotion. (a) Stimulating the CNF facilitated spontaneous locomotion. (b) Stimulating the dorsal PPN initially facilitated and then suppressed locomotion. (c) Stimulating the ventral PPN suppressed locomotion. (d) Stimulating the ventral PPN at a low intensity (20 μA) gradually increased the cycle time of locomotion. EMG activity was recorded from

the bilateral TB and Sol muscles. The treadmill speed during the recordings was 0.3 m/s. C. Effective sites from which the stimuli facilitated (open circles) and suppressed (filled circles) spontaneous locomotion. Stimulating between the two regions evoked mixed effects (hatched squares). Sites from which locomotor facilitation or suppression were elicited by stimuli with intensities at 20 μA and 30 μA are indicated by large and small symbols, respectively. SNr, substantia nigra pars reticulata.

Figure 3. Effective electrical stimulus sites in the mesopontine tegmentum
A. Effective sites on coronal (upper) and parasagittal (lower) planes for evoking muscular atonia (left column) and locomotion (right column) in 14 animals. A mixture of both was obtained from 7 animals (middle column). B. Distribution of cholinergic neurons stained by ChAT immunohistochemistry. Light microscopic photographs with lower and higher magnification are shown in the right and left columns, respectively.

Figure 4. A schematic framework of the basal ganglia - brainstem mechanisms for controlling postural muscle tone and locomotion
GABAergic neurons in the SNr of the basal ganglia project to the mesopontine tegmentum including the MLR and the PPN (Garcia-Rill, 1991; Grillner et al., 1997; Rossignol, 1996). Signals from the MLR activate the locomotor system, which is composed of the reticulospinal tract and central pattern generators (Grillner et al. 1981; Rossignol, 1996), to induce rhythmic alteration in spinal motoneurons. Cholinergic PPN neurons project to the cholinoceptive pontine inhibitory area, corresponding to the dorsomedial part of the pontine reticular formation (PRF; Mitani et al., 1988; Lai et al., 1993). The cholinoceptive PRF neurons, in turn, activate the inhibitory reticulospinal tract that provides postsynaptic inhibition of motoneurons directly, or via an inhibitory interneuron (Chase et al. 1990; Takakusaki et al., 1994). ACh, acethylcholine; GABA, γ-amino butyric acid; MLR, mesencephalic locomotor region; MNs, motoneurons; SNr, substantia nigra pars reticulata.

Figure 5. Effects of chemical stimulation of the mesopontine tegmentum

A. Effects of injecting glutamatergic agonists obtained from a single animal. (a) Schematic diagrams of the experiments. (b) Injecting NMDA into the left CNF (P 1.5, L4.0, H = –1.0) increased the bilateral soleus muscle activity. A dashed line above the recording indicates the period of the injection. (c) Commencement of the treadmill elicited locomotion. Downward and upward arrows indicate the onset and end of treadmill movements with a speed of 0.3 m/s. (d) Two hours after the first injection NMDA was also injected into the left PPN (P 2.0, L 4.5, H = –3.0) and inhibited the bilateral soleus muscle activity. Pinching the pinna after 6 min (indicated by an open triangle) restored muscle activity. (e) Approximately 1 hour after the second NMDA injection, kainic acid was injected into the right PPN (P 2.0, R 4.5, H = –2.5) and abolished muscle tone. Pinching the pinna after 5 min did not re-establish muscle tone. B. Effects of muscimol into the PPN. (a) A schematic diagram of the experiment. (b) Injecting muscimol into the left PPN alone did not alter muscle tone. (c) Effects of PPN stimulation before muscimol inhibited muscle tone. (d) – (e) However PPN stimulation after muscimol did not suppress muscle tone (d), and stimuli with a higher current and a long duration were required to reduce muscle tone (e). (f) A stimulus site and a muscimol injection site in the PPN in this animal. V, trigeminal nucleus; SO, superior olive.

Figure 6. GABAergic modulation of locomotion and muscle tone

A. (a) Tonic EMG activity of the extensor muscles. (b) Quadrupedal locomotion observed at 15 min after injecting bicuculline into the rostral CNF. (c) Another injection of bicuculline into the ventral PPN in the same cat suppressed the locomotion. A dashed line above the recording indicates the period of the injection. (d) Bicuculline injection sites in the rostral CNF and the ventral PPN. B. (a) Muscle tone suppression induced by a picrotoxin injection into the ventral PPN. Open triangles indicate stimulation applied to the left pinna by pinching the scapha. (b) Picrotoxin injection site of this animal. C. (a) A mixture of locomotion and muscle tone suppression induced by bicuculline injection into

the PPN. (b) – (c). Development of hindlimb stepping movements associated with an increase in the level of muscle tone (b) and suppression of the stepping along with a decrease in the level of muscle tone (c). (d) Bicuculline injection site.

Figure 7. Effective injection sites of GABA antagonists for evoking muscular atonia. (A), locomotion (C), and a mixture of both (B)

A. Injection sites from which muscular atonia was induced with a short latency (< 2 min, n=3) are indicated by large circles, whereas those with a longer latency (>5 min, n=3) are indicated by small circles. B. Shaded circles show injection sites from which a mixture of muscular atonia and locomotion was induced. Neither muscular atonia nor locomotion was induced by injections denoted by the open circles. C. Injections which evoked locomotion with a latency less than 10 min are indicated by large circles (n=2), whereas those with a latency more than 10 min are indicated by small circles (n=4).

Figure 8. Effects of atropine injection into the pontine reticular formation on the electrically (A) and chemically (B) -induced muscle tone suppression

A. (a) Experimental diagrams. Effects of PPN stimulation on muscle tone before (b), 10 min (c), and 40 min (d), after an atropine injection. Effects of CNF stimulation on muscle tone before (e), 12 min (f), and 42 min (g), after an atropine injection. Pontine atropine injection blocked the PPN effect, but facilitated the MLR-induced locomotor movements. (h) Stimulus sites in the CNF and the PPN, and an atropine injection site on the coronal sections of the brainstem. B. (a) An experimental diagram. (b) Muscular atonia induced by a bicuculline injection into the left PPN. Stretching the soleus muscles by extending the hindlimbs (filled triangles) did not restore muscle tone before a pontine atropine injection. The muscle tone partly recovered however when the same procedure was used after an atropine injection into the left NRPo. (c) The muscle tone was completely restored after another atropine injection into the right pontine area, in combination with muscular stretch.

Another bicuculline injection into the left PPN did not further reduce the muscle tone. (d) Bicuculline injection site in the PPN, and atropine injection sites in the bilateral PRF.

Figure 9. Nigral control of postural muscle tone

A. (a) – (b). The effects induced by the PPN (a) and SNr (b) on the postural muscle tone. (c) – (d). When conditioning SNr stimuli of 30 μA (c) and 40 μA (d) were delivered, the PPN-effect was attenuated. (e) PPN stimulation did not reduce muscle tone when SNr conditioning stimuli of 50 μA were delivered. B. (a) – (e). Effects of changes in SNr stimulus frequency. Nigral stimulus effects were prominent when the stimulus frequency was 100 Hz (c). However, either a lower (a – b) or higher (d – e) frequency of stimuli was less effective. C. (a) – (e). Changes induced by the PPN in muscle tone following conditioning stimuli which were delivered to the SNr area from dorsal (a; H = +1.0) to ventral (e; H = –3.0) locations with a vertical axis at A 3.5 and L 6.5. The stimulus sites are shown in E. SNr stimuli delivered at H = –1.0 completely abolished the PPN-effect (c). However, stimuli delivered at H = 0.0 (b) and H = –2.0 (d) partially blocked the PPN-effect. The stimuli at more dorsal (a) and ventral (e) areas did not affect the PPN-effect. D. An experimental diagram. E. Stimulus sites in the SNr (open and filled circles) area and the PPN. The maximal effect was induced at the SNr stimulus site indicated by an open circle. PAG, periaquaductal grey; RN, red nucleus.

Figure 10. Nigral control of locomotion

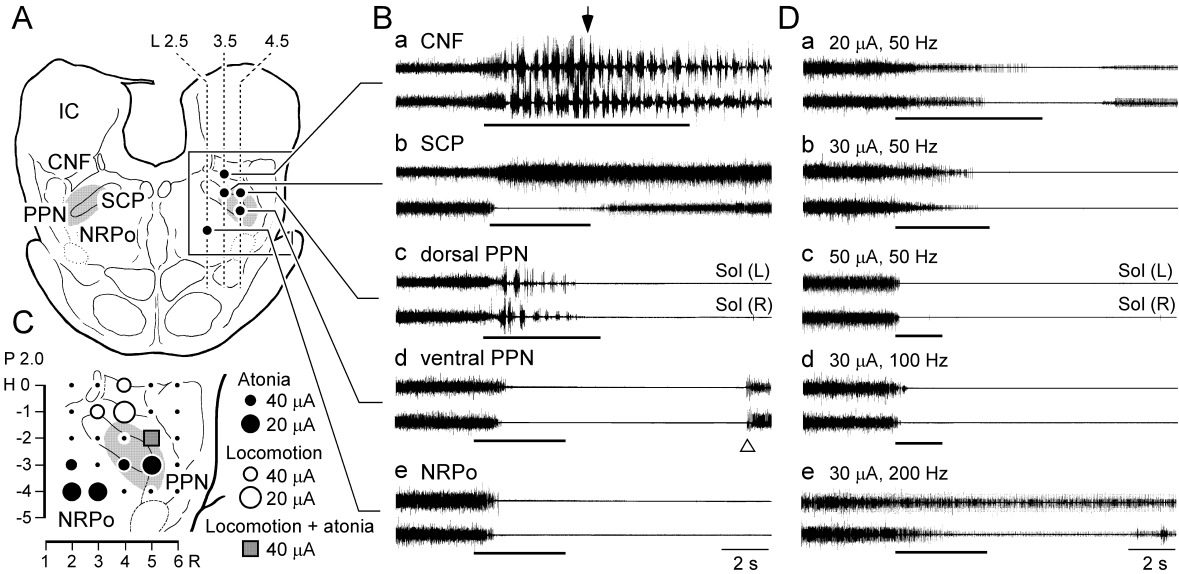
A. An experimental diagram. B. Stimulus sites of the SNr and the MLR. C. (a) SNr stimulation did not change the level of muscle tone. (b) Locomotion on a moving treadmill belt induced by the MLR. D. SNr stimulus effects on MLR-induced locomotion. Stimulation of the SNr continued throughout the recording period. From (a) to (e) the intensity of the SNr stimuli was increased from 10 μA to 50 μA. The step cycles were decreased as the stimulus intensity was increased even though the treadmill speed was kept constant. E and F. Nigral stimulus effects on locomotor parameters. E. By increasing the

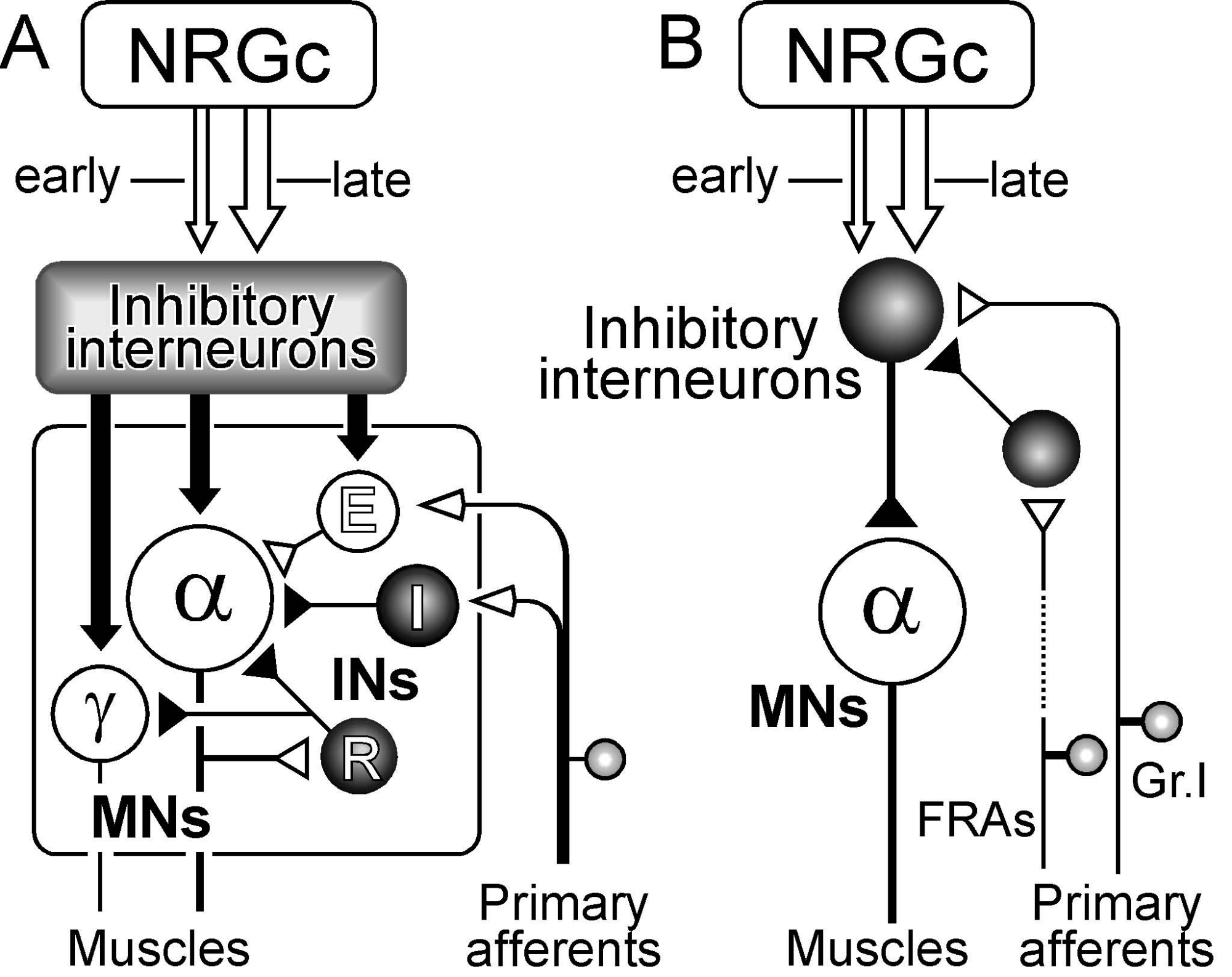
stimulus intensity, the cycle time (filled and open circles) of locomotion was increased and the locomotor onset (hatched squares) was delayed. F. Increases in cycle time and delays in locomotor onset were produced by SNr stimuli with frequencies of 50 to 100 Hz. The intensity of the SNr stimuli was kept constant at 30 μA.

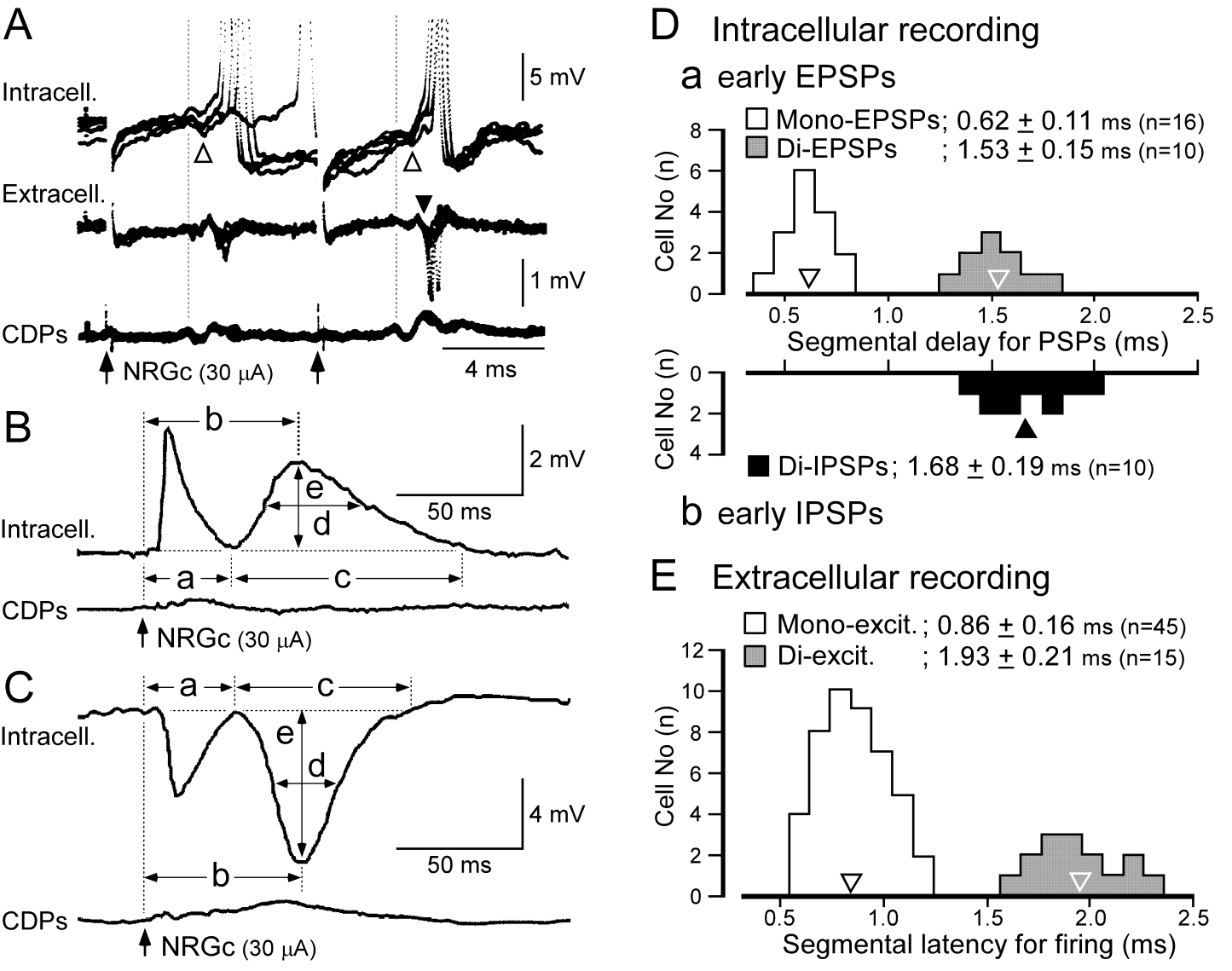
Figure 11. The effects of the SNr were blocked by bicuculline injections into the mesopontine tegmentum.
A. (a) An experimental diagram. (b) PPN-induced muscular atonia, and SNr stimulation partially restored the muscle tone. (c) A bicuculline injection (5 mM/0.25μl) into the PPN completely abolished muscle tone and SNr stimulation did not restore it. The PPN was stimulated with 30 μA and 50 Hz, and the SNr was stimulated with 50 μA and 100 Hz.
B. (a) An experimental diagram. (b) Stimulating the SNr blocked the MLR-induced locomotion. (c) However, the SNr stimuli did not block locomotion that was induced by a bicuculline injection (5 mM/0.25μl) into the CNF. The MLR was stimulated with 20 μA and 50 Hz, and the SNr was stimulated with 40 μA and 100 Hz.

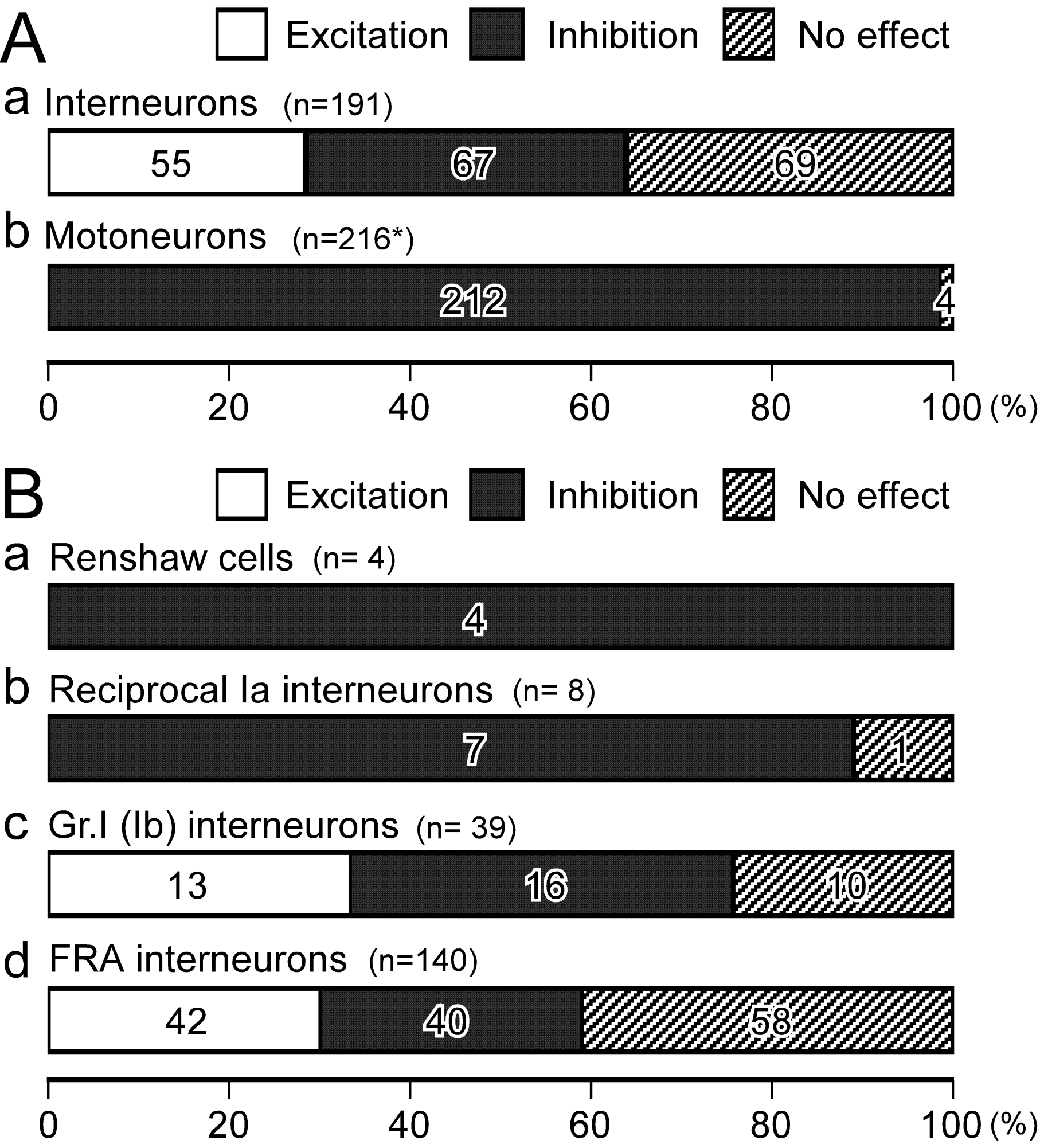
Figure 12. The effective nigral stimulus sites for blocking the PPN- (A) and MLR-effects (B). These are shown on coronal (a) and parasagittal (b) planes.
A. (a) – (b) The large circles indicate stimulus sites where the stimulation completely blocked the PPN-effect. The small circles indicate sites where SNr stimulation attenuated the PPN-effect. In both (a) and (b) the SNr was stimulated with 50 μA and 100 Hz, and the PPN was stimulated with 20 μA and 50 Hz. B. (a) – (b) SNr stimulation at the sites indicated with large circles abolished locomotion. The small circles indicate sites where SNr stimulation decreased the step cycles. In (a) the SNr was stimulated with 40 μA and 100 Hz, and the MLR was stimulated with 15 μA and 50 Hz. In (b) the SNr was stimulated with 50 μA and 100 Hz and the MLR was stimulated with 20 μA and 50 Hz. CP, cerebral peduncle; SNc, substantial nigra pars compacta.

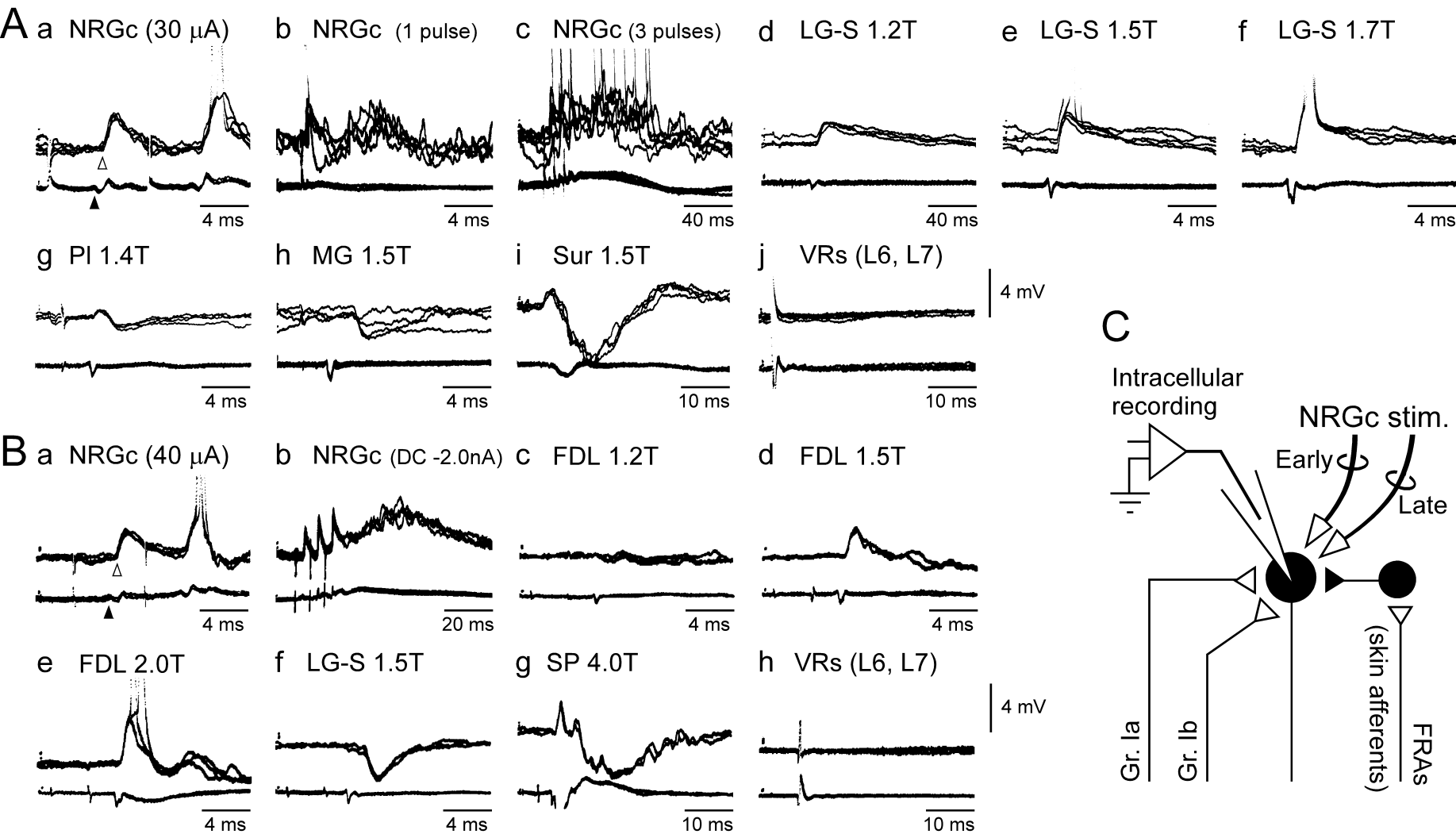
Figure 13. A hypothetical model for the control of movement by the basal ganglia A. A GABAergic projection from the medial SNr to the MLR controls locomotion and another projection from the lateral SNr to the PPN controls postural muscle tone. B. The left abscissa indicates the amount of movement that is determined by the cortico-basal ganglia loop. The right abscissa indicates the locomotor patterns that can be determined by the medial SNr projection to the MLR. The ordinate indicates the level of muscle tone that can be determined by the lateral SNr projection to the PPN. In normal conditions the value of the degrees of freedom for output of the basal ganglia is quite large. Therefore the amount of movement, the locomotor pattern, and the level of muscle tone, would vary considerably as indicated by the square. C. Excessive basal ganglia output may produce a decrease of movement (hypokinesia), gait disturbances, and an increase in muscle tone (hypertonus, rigidity) as expressed in Parkinson's disease (a). In contrast, a decrease in the basal ganglia output may result in a hyperkinesia-hypotonus syndrome such as Huntington's disease (b). CTX, cerebral cortex; GPi, internal segment of the globus pallidus; MNs, motoneurons.

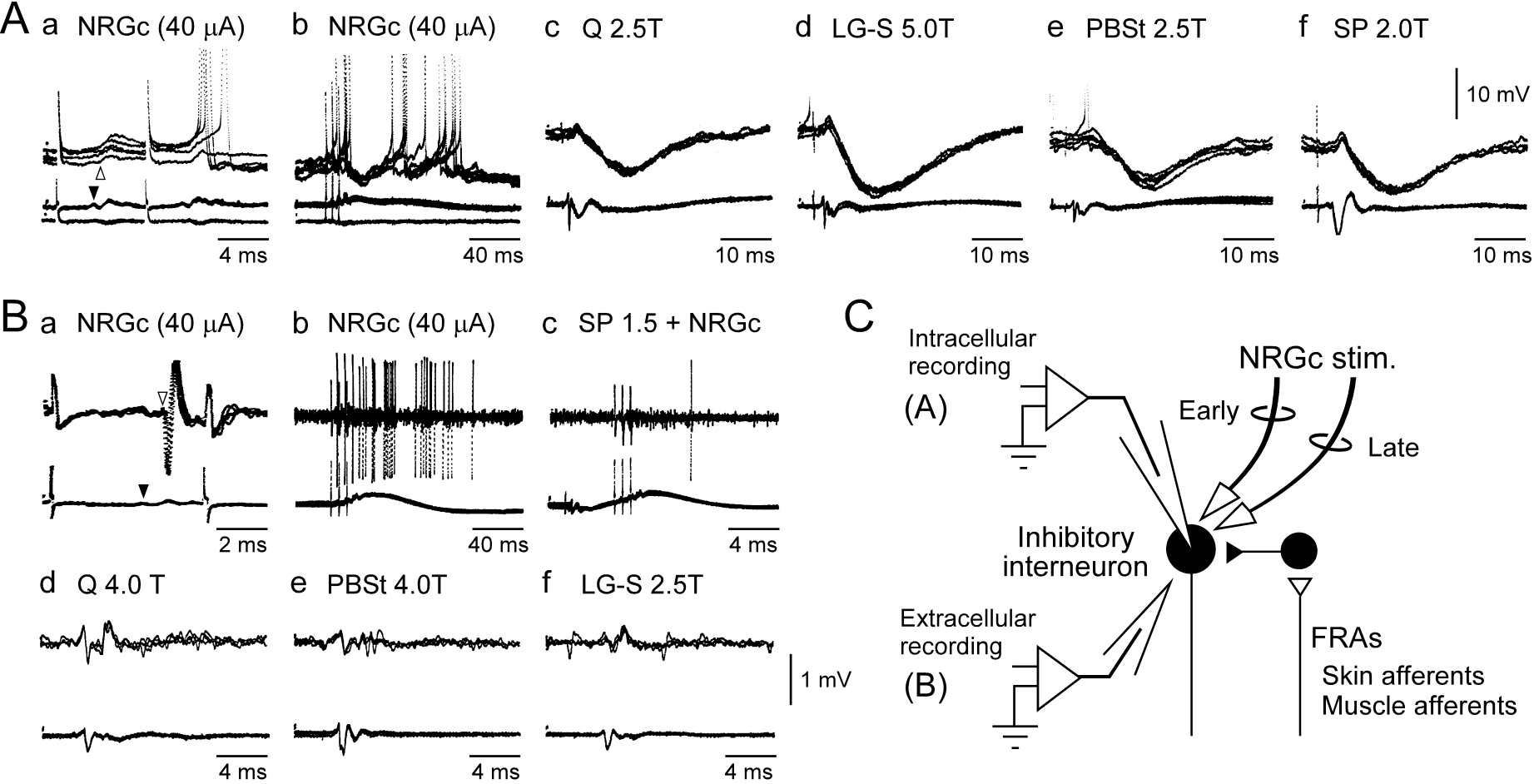

A
L 2.5
3.5
4.5
IC
CNF
SCP
PPN
NRPo
B
a CNF
b SCP
c dorsal PPN
Sol (L)
Sol (R)
d ventral PPN
e NRPo
2 s
D
a 20 μA, 50 Hz
b 30 μA, 50 Hz
c 50 μA, 50 Hz
Sol (L)
Sol (R)
d 30 μA, 100 Hz
e 30 μA, 200 Hz
2 s
C
P 2.0
H 0
-1
-2
-3
-4
-5
PPN
NRPo
1 2 3 4 5 6 R
Atonia
40 μA
20 μA
Locomotion
40 μA
20 μA
Locomotion + atonia
40 μA

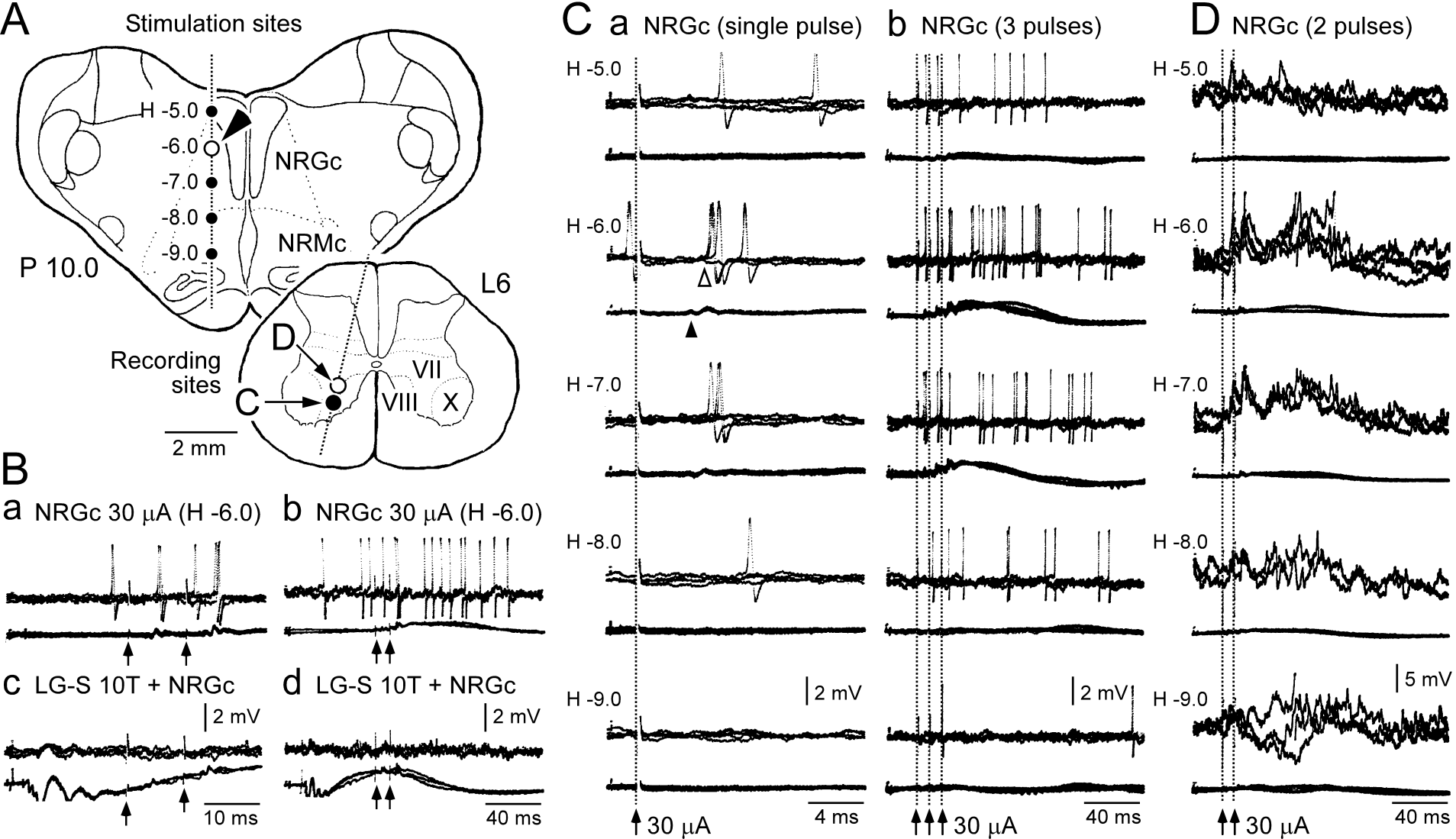

A
NRGc
early
late
Inhibitory interneurons
E
α
I
INs
γ
R
MNs
Muscles
Primary afferents
B
NRGc
early
late
Inhibitory interneurons
α
MNs
FRAs
Gr.I
Muscles
Primary afferents

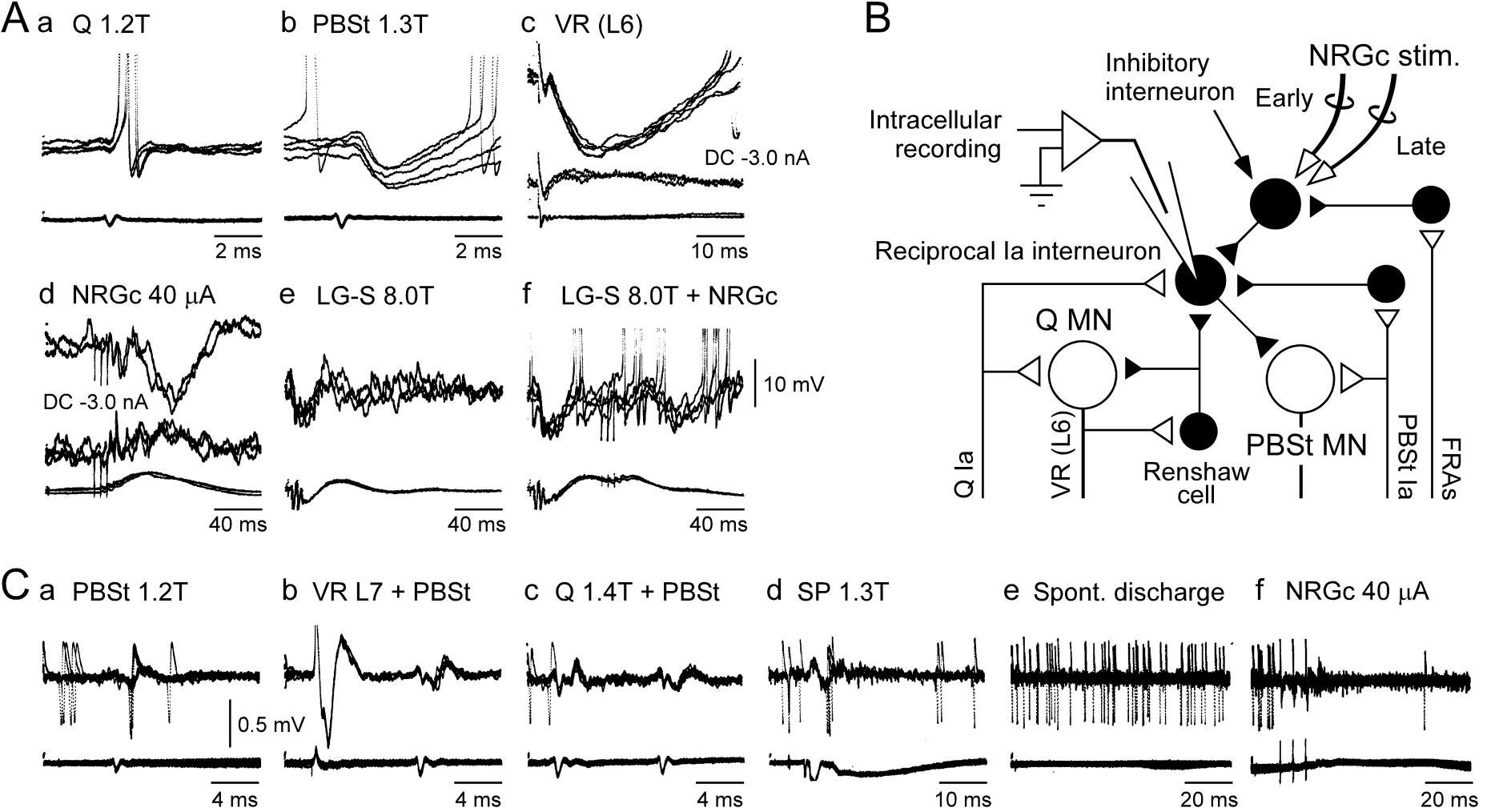

A
Intracell.
5 mV
Extracell.
1 mV
CDPs
NRGc (30 μA)
4 ms
B
b
e
d
2 mV
50 ms
Intracell.
a
c
CDPs
NRGc (30 μA)
C
a
c
Intracell.
e
d
4 mV
50 ms
b
CDPs
NRGc (30 μA)
D Intracellular recording
a early EPSPs
Mono-EPSPs; 0.62 ± 0.11 ms (n=16)
Di-EPSPs ; 1.53 ± 0.15 ms (n=10)
Cell No (n)
Segmental delay for PSPs (ms)
Di-IPSPs; 1.68 ± 0.19 ms (n=10)
b early IPSPs
E Extracellular recording
Mono-excit. ; 0.86 ± 0.16 ms (n=45)
Di-excit. ; 1.93 ± 0.21 ms (n=15)
Cell No (n)
Segmental latency for firing (ms)

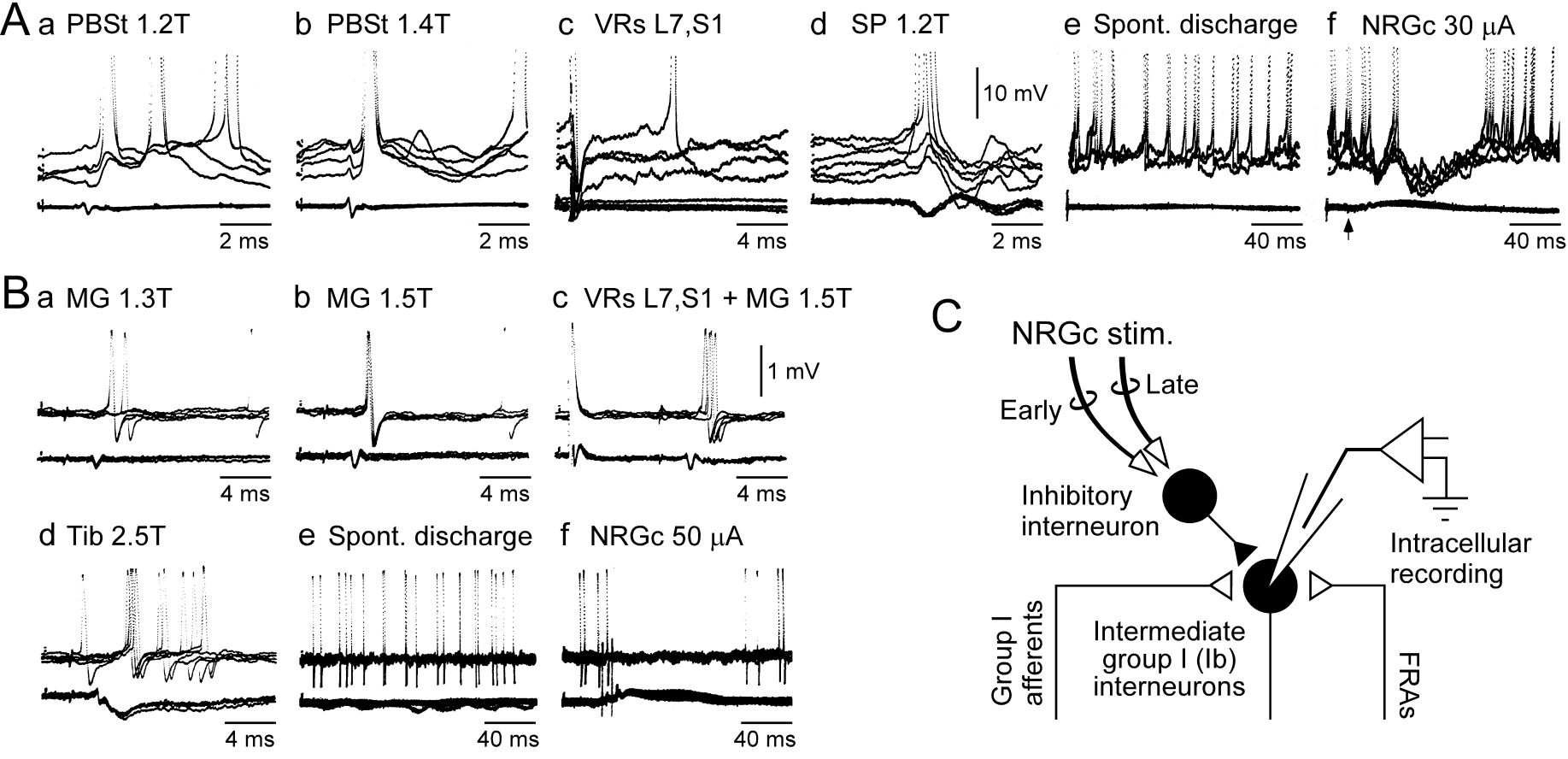

A
Excitation
Inhibition
No effect
a Interneurons (n=191)
55
67
69
b Motoneurons (n=216*)
212
4
0
20
40
60
80
100 (%)
B
Excitation
Inhibition
No effect
a Renshaw cells (n= 4)
4
b Reciprocal Ia interneurons (n= 8)
7
1
c Gr.I (Ib) interneurons (n= 39)
13
16
10
d FRA interneurons (n=140)
42
40
58
0
20
40
60
80
100 (%)

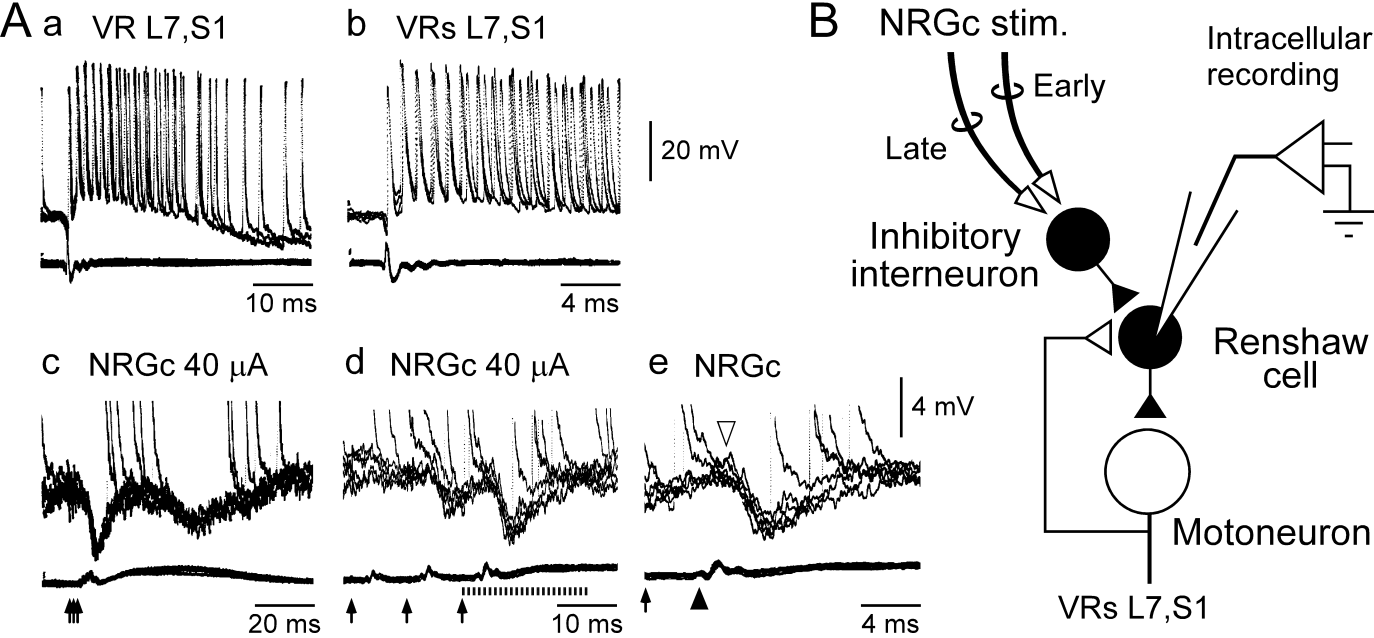

A
a NRGc (30 μA)
b NRGc (1 pulse)
c NRGc (3 pulses)
d LG-S 1.2T
e LG-S 1.5T
f LG-S 1.7T
4 ms
4 ms
40 ms
40 ms
4 ms
4 ms
g PI 1.4T
h MG 1.5T
i Sur 1.5T
j VRs (L6, L7)
4 mV
4 ms
4 ms
10 ms
10 ms
B
a NRGc (40 μA)
b NRGc (DC -2.0nA)
c FDL 1.2T
d FDL 1.5T
4 ms
20 ms
4 ms
4 ms
e FDL 2.0T
f LG-S 1.5T
g SP 4.0T
h VRs (L6, L7)
4 mV
4 ms
4 ms
10 ms
10 ms
C
Intracellular recording
NRGc stim.
Early
Late
Gr. Ia
Gr. Ib
(skin afferents)
FRAs

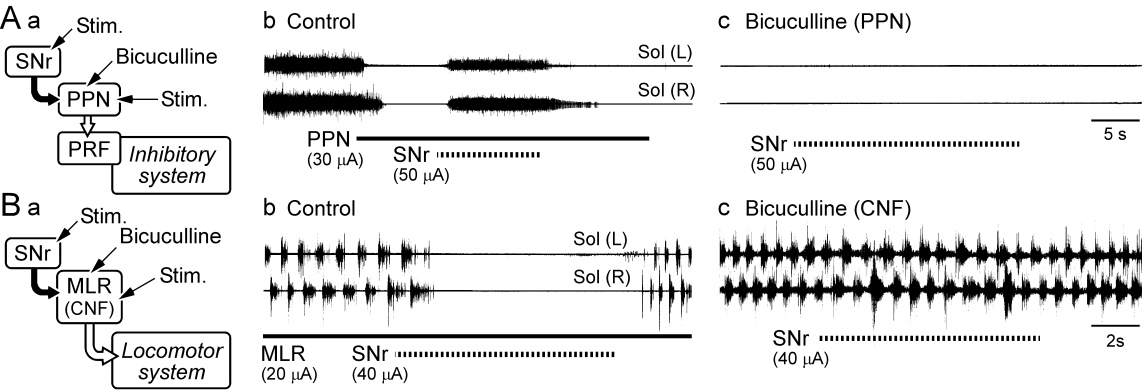

A
a NRGc (40 μA)
b NRGc (40 μA)
c Q 2.5T
d LG-S 5.0T
e PBSt 2.5T
f SP 2.0T
10 mV
4 ms
40 ms
10 ms
10 ms
10 ms
10 ms
B
a NRGc (40 μA)
b NRGc (40 μA)
c SP 1.5 + NRGc
2 ms
40 ms
4 ms
d Q 4.0 T
e PBSt 4.0T
f LG-S 2.5T
1 mV
4 ms
4 ms
4 ms
C
Intracellular recording
(A)
NRGc stim.
Early
Late
Inhibitory interneuron
Extracellular recording
(B)
FRAs
Skin afferents
Muscle afferents

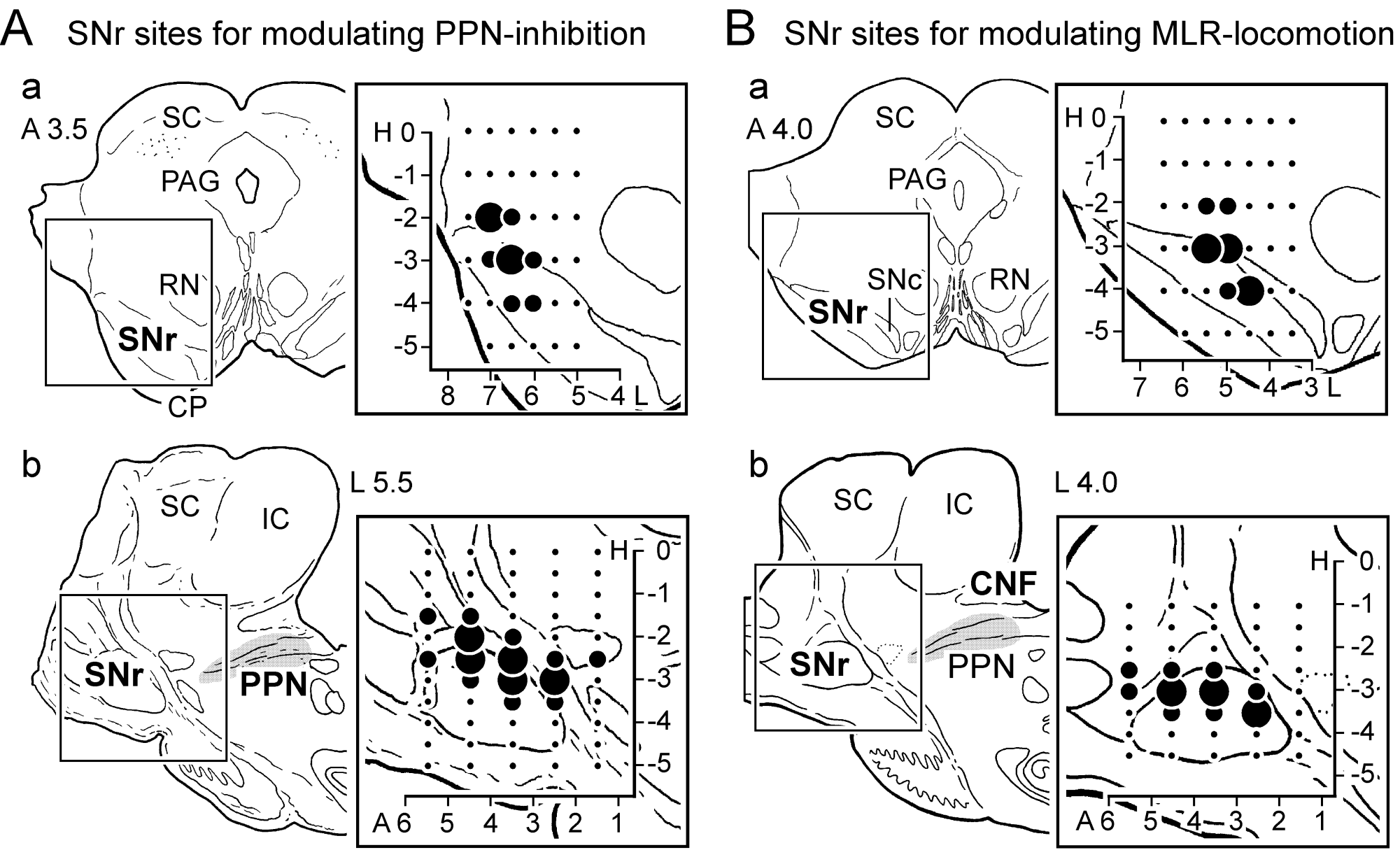

A
Stimulation sites
H -5.0
-6.0
-7.0
-8.0
-9.0
NRGc
NRMc
P 10.0
L6
D
C
Recording sites
VII
VIII
X
2 mm
B
a NRGc 30 μA (H -6.0)
b NRGc 30 μA (H -6.0)
c LG-S 10T + NRGc
d LG-S 10T + NRGc
2 mV
10 ms
40 ms
C
a NRGc (single pulse)
b NRGc (3 pulses)
D NRGc (2 pulses)
H -5.0
H -6.0
H -7.0
H -8.0
H -9.0
Δ
2 mV
5 mV
30 μA
4 ms
40 ms

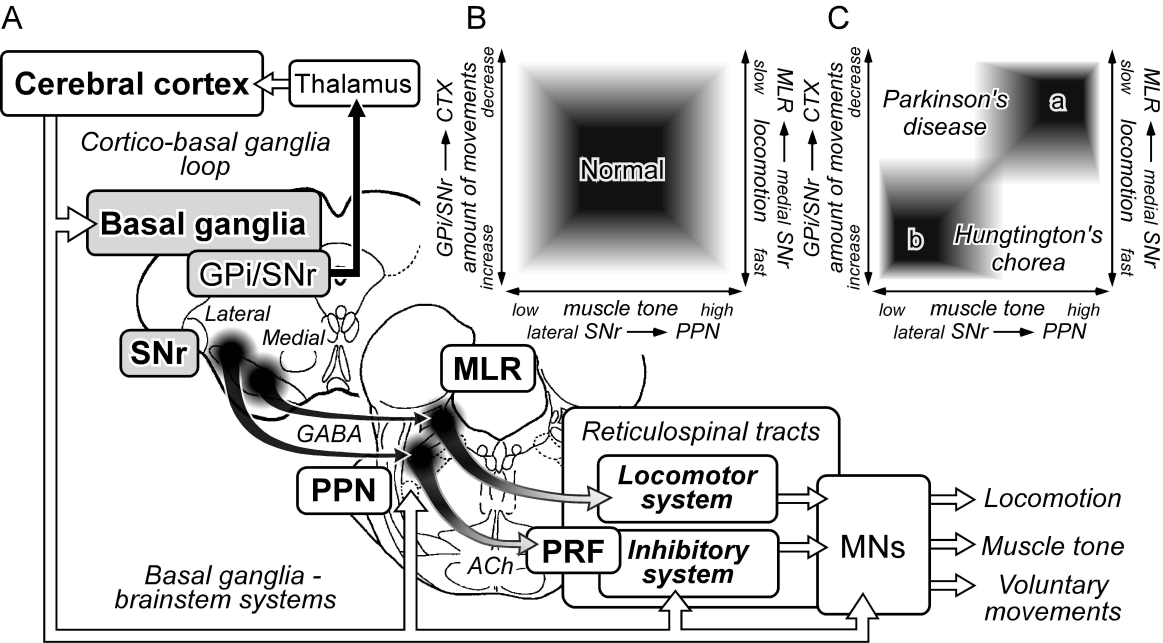

A
a Q 1.2T
b PBSt 1.3T
c VR (L6)
DC -3.0 nA
2 ms
2 ms
10 ms
d NRGc 40 μA
e LG-S 8.0T
f LG-S 8.0T + NRGc
DC -3.0 nA
10 mV
40 ms
40 ms
40 ms
B
Inhibitory interneuron
NRGc stim.
Early
Late
Intracellular recording
Reciprocal Ia interneuron
Q MN
Q Ia
VR (L6)
Renshaw cell
PBSt MN
PBSt Ia
FRAs
C
a PBSt 1.2T
b VR L7 + PBSt
c Q 1.4T + PBSt
d SP 1.3T
e Spont. discharge
f NRGc 40 μA
0.5 mV
4 ms
4 ms
4 ms
10 ms
20 ms
20 ms


A
a PBSt 1.2T
b PBSt 1.4T
c VRs L7,S1
d SP 1.2T
e Spont. discharge
f NRGc 30 μA
10 mV
2 ms
2 ms
4 ms
2 ms
40 ms
40 ms
B
a MG 1.3T
b MG 1.5T
c VRs L7,S1 + MG 1.5T
1 mV
4 ms
4 ms
4 ms
d Tib 2.5T
e Spont. discharge
f NRGc 50 μA
4 ms
40 ms
40 ms
C
NRGc stim.
Early
Late
Inhibitory interneuron
Intracellular recording
Group I afferents
Intermediate group I (Ib) interneurons
FRAs


A
a VR L7,S1
b VRs L7,S1
20 mV
10 ms
4 ms
c NRGc 40 μA
d NRGc 40 μA
e NRGc
4 mV
20 ms
10 ms
4 ms
B
NRGc stim.
Early
Late
Intracellular recording
Inhibitory interneuron
Renshaw cell
Motoneuron
VRs L7,S1


A a
Stim.
SNr
Bicuculline
PPN
Stim.
PRF
Inhibitory system
b Control
Sol (L)
Sol (R)
PPN
(30 μA)
SNr
(50 μA)
c Bicuculline (PPN)
SNr
(50 μA)
5 s
B a
Stim.
SNr
Bicuculline
MLR
(CNF)
Stim.
Locomotor system
b Control
Sol (L)
Sol (R)
MLR
(20 μA)
SNr
(40 μA)
c Bicuculline (CNF)
SNr
(40 μA)
2s


A SNr sites for modulating PPN-inhibition
a
A 3.5
SC
PAG
RN
SNr
CP
H 0
-1
-2
-3
-4
-5
8 7 6 5 4 L
b
SC
IC
L 5.5
SNr
PPN
H 0
-1
-2
-3
-4
-5
A 6 5 4 3 2 1
B SNr sites for modulating MLR-locomotion
a
A 4.0
SC
PAG
SNc
SNr
RN
H 0
-1
-2
-3
-4
-5
7 6 5 4 3 L
b
SC
IC
L 4.0
CNF
SNr
PPN
H 0
-1
-2
-3
-4
-5
A 6 5 4 3 2 1


A
Cerebral cortex
Thalamus
Cortico-basal ganglia loop
Basal ganglia
GPi/SNr
Lateral
Medial
SNr
MLR
GABA
PPN
Reticulospinal tracts
Locomotor system
PRF
Inhibitory system
ACh
MNs
Locomotion
Muscle tone
Voluntary movements
Basal ganglia - brainstem systems
B
GPi/SNr → CTX
amount of movements
decrease
increase
Normal
MLR → medial SNr
slow
locomotion
fast
low
muscle tone
high
lateral SNr → PPN
C
Parkinson's disease
a
b
Hungtington's chorea