

Cessation of activity in red nucleus neurons during stimulation of the medial medulla in decerebrate rats

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The pontine oral reticular nucleus, gigantocellular reticular nucleus (Gi) and dorsal paragigantocellular nucleus (DPGi) of the medulla are key elements of a brainstem-reticulospinal inhibitory system that participates in rapid eye movement (REM) sleep atonia. Our recent study has shown that excitation of these brainstem nuclei in decerebrate rats inhibits locus coeruleus cells and the midbrain locomotor region neurons related to muscle tone facilitation. In the present study we have examined the influences of electrical and chemical stimulation of Gi and DPGi inhibitory sites on the activity of neurons located in the magnocellular part of the red nucleus (RMC), a cell group that participates in both the tonic and phasic regulation of motor output. A total of 192 RMC neurons were recorded in precollicular–premamillary decerebrate rats with muscle rigidity and induced locomotion. Thirty-three RMC neurons were identified antidromically as rubrospinal (RMC-spinal) cells by stimulation of the contralateral dorsolateral funiculus at the L2 level. A total of 141 RMC neurons (88.7%) and all RMC-spinal neurons were inhibited during electrical stimulation of Gi and DPGi inhibitory sites. This cessation of activity was correlated with bilateral muscle atonia or blockage of locomotion. Six RMC cells (3.8%) were excited ($224 \pm 50\%$, $n = 6$, minimum = 98, maximum = 410, $P < 0.05$) and 11 cells (7%) gave no response to Gi and DPGi stimulation. Microinjections of kainic acid (100 μM , 0.2 μl) into Gi and DPGi inhibitory sites, previously identified by electrical stimulation, produced a short-latency (35 ± 3.5 s, $n = 11$) decrease of rigid hindlimb muscle tone and inhibition of all tested RMC ($n = 7$) and RMC-spinal ($n = 5$) neurons. These results, combined with our recent published data, suggest that inhibition of motor function during activation of the brainstem inhibitory system is related to both the descending inhibition of spinal motoneurons and suppression of activity in supraspinal motor facilitatory systems. These two mechanisms acting synergistically may cause generalized motor inhibition during REM sleep and cataplexy.

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Inhibition of evoked locomotor activity and muscle tone in intact, anaesthetized and decerebrate animals may be elicited by electrical and chemical stimulation of the medial and dorsolateral regions of the pons, as well as the medial division of the medulla (Magoun & Rhines, 1946; Mori *et al.* 1978; Lai & Siegel, 1988; Mileikovskii *et al.* 1991; Oka *et al.* 1993). It is generally agreed that this inhibition is related to excitation of a descending reticulospinal system, which in turn hyperpolarizes spinal alpha motoneurons (Jankowska *et al.* 1968; Takakusaki *et al.* 1989, 1994). However, recent studies have shown that excitation of brainstem inhibitory regions also produces a marked reduction of activity in some supraspinal motor systems, which participate in facilitation of muscle tone and locomotion (Iwakiri *et al.* 1995; Mileikovskii *et al.* 2000; Mileykovskiy *et al.* 2000). We propose that excitation of brainstem inhibitory structures evokes generalized

suppression in the brainstem motor facilitatory systems, and this suppression acts synergistically with inhibition produced by the descending inhibitory reticulospinal system. The red nucleus (RN) is a prominent structure in the motor system of mammals and consists of posterior magnocellular (RMC) and anterior parvocellular parts. RMC neurons form the rubrospinal tract and participate in the production of skilled forelimb movements (Martin & Ghez, 1991; Martin *et al.* 1993; Jarratt & Hyland, 1999; van Kan & McCurdy, 2001), regulation of locomotion (Orlovsky 1972; Arshavsky *et al.* 1988; Ruigrok *et al.* 1996; Rho *et al.* 1999; Muir & Wishaw, 2000) and coordination of the motor responses to pain (Matsumoto & Walker, 1991; Horn *et al.* 1998).

The medial parts of the gigantocellular reticular nucleus (Gi) and dorsal paragigantocellular reticular nucleus (DPGi) are the main elements of the reticulospinal

inhibitory system responsible for the hyperpolarization of spinal alpha motoneurons (Takakusaki *et al.* 1989; 1994; Chase & Morales, 1990). In the present study we have used precollicular-premamillary decerebrate rats to determine the responses of unidentified RMC and RMC-spinal neurons, which are related to hindlimb muscle activity, to electrical and chemical stimulation of Gi and DPGi sites that suppress muscle tone and block locomotion.

METHODS

Surgery

We used a precollicular-premamillary preparation to study the influence of medullary inhibitory site stimulation upon the discharge patterns of the RN neurons that control both the phasic parameters of movement and static torque. All procedures were approved by the Animal Studies Committee of the Sepulveda VA Medical Center/UCLA, in accordance with US Public Health Service guidelines. Animals were anaesthetized with halothane (2.5% halothane plus oxygen) followed by ketamine HCl (Ketalar, 70 mg kg⁻¹) for cannulation of the trachea and decerebration. Two holes (diameter 1 mm) were drilled over the skull above the RMC at 6 mm posterior to bregma and 0.7 mm lateral to the midline for insertion of unit recording electrodes. One hole (diameter 2.0 mm) was drilled above the Gi and DPGi at 11.0 mm posterior to bregma for the insertion of stimulating electrodes or injection cannulae. The coordinates of all structures were based on the atlas of Paxinos & Watson (1997).

Two rectangular holes were cut in each parietal bone in preparation for decerebration. The transverse anterior and posterior borders of these holes were located 1 and 5 mm posterior to bregma, respectively, with the longitudinal sagittal border 0.2 mm from the midline. Precollicular-premamillary decerebration was performed 3.5–4.0 mm posterior to bregma with a stainless steel spatula, taking care not to injure the sagittal vein. The forebrain was completely removed and excess fluid was aspirated by syringe and absorbed with Gelfoam. A cotton tampon moistened with saline and 2% lidocaine was then inserted into the skull in place of the forebrain. The level of transection was verified by histological analysis and plotted according to the rat brain atlas (Paxinos & Watson, 1997).

Three insulated stainless steel wires (diameter 62 μm) were implanted into the spinal cord to stimulate the right and left dorsolateral funiculus at the L2 level. Wires were fixed with a specially designed clamp and dental acrylic. Pressure points on the head were infiltrated with lidocaine (4%). Anaesthesia was then discontinued. Eighteen Wistar rats (250–300 g) were operated on, of which eight showed bilateral muscle rigidity after precollicular-premamillary decerebration, with EMG amplitudes ranging from 50 to 400 μV . Five rats responded with 30–180 s of repetitive stepping behaviour after exteroceptive stimulation (pinching or touching of the back, tail or hindlimbs).

Stimulation and recording

Electrical stimulation of Gi and DPGi sites (0.2 ms; 50 Hz; 30–250 μA , continuous stimulation 10–15 s) via a bipolar stimulating electrode (stainless steel, 100 μm , tip separation 80–100 μm) was used to produce muscle atonia and locomotor and withdrawal reflex suppression. To select Gi and DPGi inhibitory sites, electrical stimulation was performed during electrode insertion in 0.3 mm steps. Bipolar stimulating electrodes

were used to reduce electrical artefacts during unit recording. Stimulation artefacts were determined to have a duration of less than 1.4 ms. Electrical stimuli were delivered with the aid of a S88 stimulator (Grass Instrument) and Grass SIU5 stimulus isolation unit. Extracellular unit recordings were performed using tungsten monopolar microelectrodes (A-M Systems, Carlborg, WA, USA). The indifferent electrode was placed in the frontal cranial bone. Spikes were amplified with a model 1700A-M Systems amplifier. Identification of RMC neurons was based on electrophysiological characteristics, as reported previously (Eccles *et al.* 1975; Gibson *et al.* 1985; van Kan & McCurdy 2001): (1) action potentials of large amplitude (0.4–2 mV), (2) a first spike wave with a long duration (0.5–1.0 ms) and (3) background activity that was modulated during movements of contralateral body parts. RMC cells were determined to be rubrospinal (RMC-spinal) neurons related to hindlimb muscle activity, if units met the following criteria: (1) stimulation of the contralateral dorsolateral funiculus at the L2 level (0.2 ms, 50–300 μA , trains of three pulses at a frequency of > 200 Hz) produced antidromic spikes at an invariant latency of < \pm 0.2 ms and (2) collision of antidromic spikes with spontaneous spikes could be demonstrated.

EMG electrodes (stainless steel wires, 100 μm) were implanted bilaterally into the semimembranosus muscles (Sm), tibialis anterior muscles (Ta), sartorius muscles (Srt), and gastrocnemius muscles (Gc). EMG activity was amplified using a Grass polygraph (model 78D). Unit pulses and EMG were recorded on a personal computer using the 1401 plus interface and Spike 2 program (Cambridge Electronic Design, Cambridge, UK). The rate of digitization was 372 Hz for the EMG and 21 kHz for unit activity.

Microinjections of kainic acid

To exclude the possibility that excitation of axons of passage was responsible for the effects of Gi and DPGi stimulation, microinjections of kainic acid (KA) (100 μM , 0.2 μl) were performed into Gi inhibitory sites ($n = 11$). These sites were previously identified by electrical stimulation (0.2 ms, 50–200 μA , 50 Hz, continuous 10–15 s) via tungsten microelectrodes (A-M Systems, Carlborg, WA, USA) as points producing bilateral hindlimb muscle atonia. KA was dissolved in 0.9% saline to obtain the required concentration. Control saline microinjections ($n = 5$) into Gi and DPGi inhibitory sites were carried out in two rats. A 1 μl Hamilton microsyringe and injecting cannulae with an outside tip diameter of 0.2 mm were used for all microinjections.

Histology

Cathodal current (30–50 μA , 10–30 s) was passed through the microelectrodes at the end of each recording track. Rats were deeply anaesthetized with pentobarbital (70 mg kg⁻¹, i.p.) and perfused transcardially with 0.01 M PBS, pH 7.4, followed by 4% paraformaldehyde in 0.1 M PBS. Brains were removed and cut into 60 μm sections. The location of recorded neurons was determined by using the track made by the microelectrode, the depth of the marking lesion and the depth measurements on the microdrive. The electrode tracks and marking lesions were visualized with the aid of a Nikon microscope and plotted with a NeuroLucida interface according to the rat brain atlas (Paxinos & Watson, 1997). Cannula tracks were visualized using a Leica MZ6 microscope.

Data analysis

Unit firing rates were analysed using the Wilcoxon matched-pairs test. The average unit firing rates were calculated for 10 s before and during Gi and DPGi electrical or chemical stimulation in animals with muscle rigidity and for 0.2 s in the 10 swing and

stance phases of the locomotor cycle. The latency of muscle tone suppression was measured from the start of electrical stimulation or microinjection to a 50% decrease in EMG amplitude compared with baseline. The duration of suppression was calculated from the time of onset of a decrease in muscle tone to return of the tone to 50% of the baseline level. The latencies and durations of muscle tone suppression were averaged for right and left hindlimbs for each electrical stimulation and microinjection. All values are given as the mean \pm S.E.M.

RESULTS

Electrophysiological characteristics of RMC and RMC-spinal neurons

A total of 159 RMC and 33 RMC-spinal neurons were recorded in eight rats with bilateral muscle rigidity and five rats that showed stepping in response to exteroceptive stimulation. The duration of the first spike wave in RMC and RMC-spinal neurons was 0.66 ± 0.01 ms ($n = 192$, minimum (min) = 0.51 ms, maximum (max) = 1.0 ms). Amplitudes of RMC spikes ranged between 0.4 mV and 1.0 mV. RMC-spinal neurons that send projections to

lumbar levels were located mainly in the ventrolateral part of the RMC (Figs 1 and 5) and responded antidromically upon stimulation of the contralateral dorsolateral funiculus at the L2 level with a latency ranging from 2.2 ms to 6.2 ms and a mean of 3.6 ± 0.2 ms ($n = 33$).

Animals with rigid muscle tone

RMC-spinal neurons. The firing rates of 21 RMC-spinal neurons (Fig. 1) were analysed during electrical stimulation (0.2 ms, 50 Hz, 50–250 μ A) of Gi and DPGi sites producing bilateral muscle atonia in rats with muscle rigidity (Fig. 2). RMC-spinal neurons had an average firing rate of 28 ± 3 spikes s^{-1} ($n = 21$, min = 5 spikes s^{-1} , max = 63 spikes s^{-1}) during muscle rigidity. Electrical stimulation of Gi and DPGi inhibitory sites produced bilateral suppression of muscle tone and inhibition of all RMC-spinal neurons (Fig. 3A). Sixteen RMC-spinal neurons were completely inhibited and the discharge of five cells was reduced by $79.2 \pm 5.0\%$ compared with the baseline ($n = 5$, min = 63%, max = 92%, $P < 0.05$). Inhibition of RMC-spinal neurons preceded the reduction in muscle tone

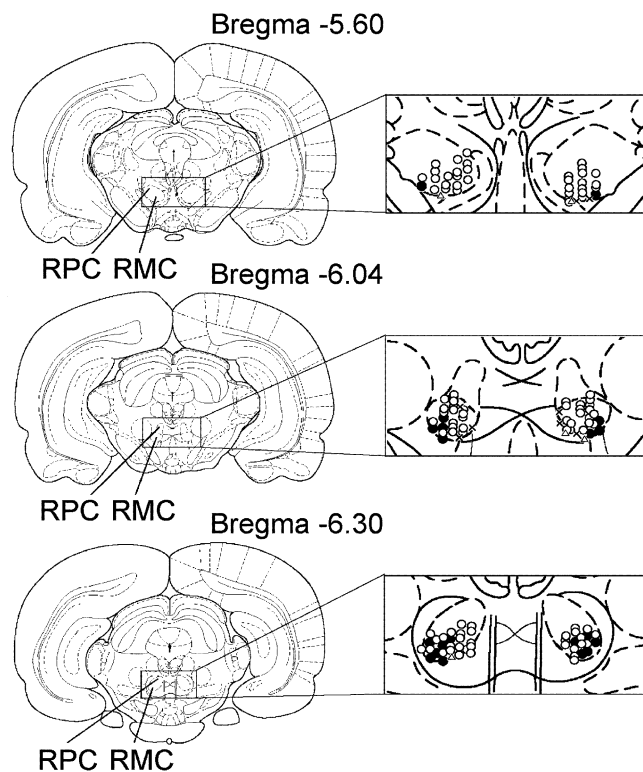


Figure 1. Coronal views of the rat midbrain showing the location of recorded magnocellular red nucleus (RMC) and rubrospinal RMC (RMC-spinal) neurons

Open and black circles indicate RMC and RMC-spinal neurons, respectively, which were inhibited by Gi and DPGi stimulation, producing bilateral muscle atonia. Triangles indicate RMC neurons that were excited by Gi and DPGi stimulation. Crosses indicate RMC neurons that were unaffected by Gi and DPGi stimulation. RPC, parvocellular part of the red nucleus.

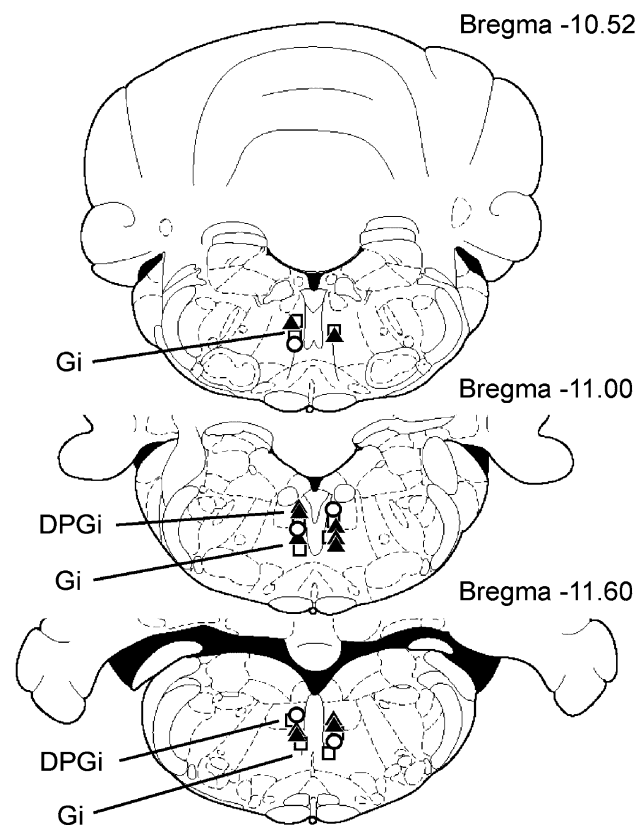


Figure 2. Location of gigantocellular reticular nucleus (Gi) and dorsal paragigantocellular nucleus (DPGi) sites that produced bilateral muscle atonia, blockage of locomotion and the withdrawal reflex

Squares and triangles indicate Gi and DPGi sites that produced bilateral muscle atonia during electrical stimulation ($< 100 \mu$ A) and microinjections of kainic acid (100 μ M, 0.2 μ l), respectively. Circles indicate Gi and DPGi sites that suppressed locomotion and the withdrawal reflex.

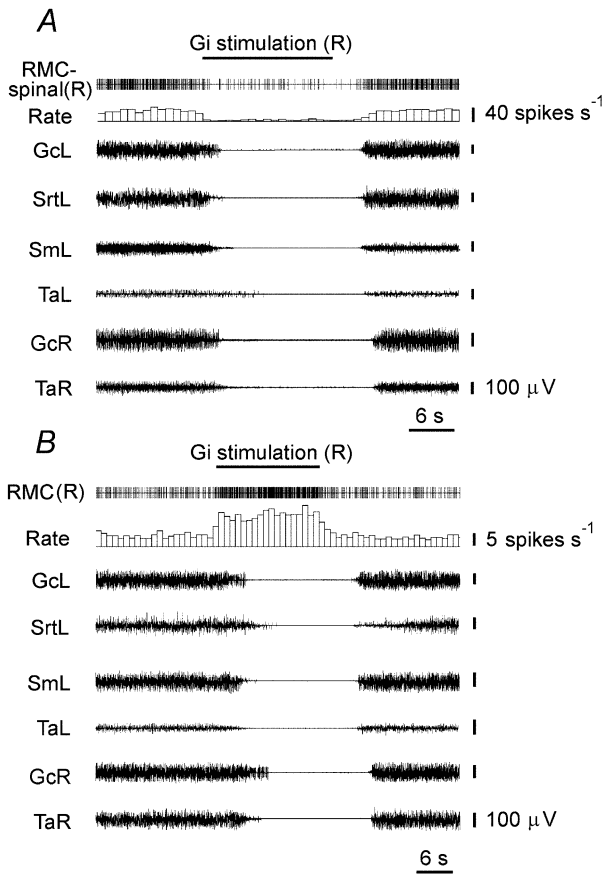


Figure 3. RMC-spinal neuron inhibition (A) and RMC neuron excitation (B) during Gi electrical stimulation inducing bilateral muscle atonia

RMC-spinal(R), RMC-spinal neuron (right side); RMC(R), RMC neuron (right side); GcL, GcR, EMG of gastrocnemius muscle, left side and right side, respectively; SmL, EMG of the semimembranosus muscle (left side); SrtL, EMG of sartorius muscle (left side); TaL, TaR, EMG of the tibialis anterior muscle, left side and right side, respectively. Stimulating currents were $< 100 \mu\text{A}$.

during Gi and DPGi stimulation by an average of $1.5 \pm 0.2 \text{ s}$ ($n = 21$, min = 0.3 s, max = 3.5 s) and resumption of the baseline firing rate in these cells preceded the recovery of muscle tone by an average of $1.6 \pm 0.2 \text{ s}$ ($n = 21$, min = 0.5 s, max = 3.8 s).

RMC neurons. The activity of 117 RMC neurons (Fig. 1) was analysed during stimulation ($50\text{--}180 \mu\text{A}$) of Gi and DPGi sites producing bilateral hindlimb muscle atonia. The RMC cells could be divided into three groups based on their responses. The first group ($n = 104$) consisted of RMC neurons that were depressed by Gi and DPGi stimulation. Forty-two RMC neurons ceased discharge completely and the firing rate of 62 cells was reduced by $68.7 \pm 2.2 \%$ during stimulation ($n = 62$, min = 37%, max = 93%, $P < 0.0001$). Reduction of activity in these neurons during Gi and DPGi stimulation preceded muscle tone suppression by an average of $2.4 \pm 0.2 \text{ s}$ ($n = 104$, min = 0.3 s, max = 8.7 s) and firing rate resumption preceded the muscle tone recovery after stimulation by an average of $3.1 \pm 0.3 \text{ s}$ ($n = 104$, min = 0.1 s, max = 13.5 s). The average firing rate of RMC neurons in this group was $24.9 \pm 1.4 \text{ spikes s}^{-1}$ ($n = 104$, min = 6 spikes s^{-1} , max = 62 spikes s^{-1}) during muscle rigidity. Neurons in the second group ($n = 5$) were excited by stimulation of Gi and DPGi inhibitory sites (Fig. 3B). These RMC cells had an average firing rate of $3.7 \pm 0.5 \text{ spikes s}^{-1}$ during muscle rigidity ($n = 5$, min = 2.5 spikes s^{-1} , max = 5 spikes s^{-1}). The discharge rate of these cells increased by an average of $249 \pm 54 \%$ ($n = 5$, min = 98%, max = 410%, $P < 0.05$) during Gi and DPGi stimulation compared with the baseline. RMC neurons in the third group ($n = 8$) did not respond to stimulation of Gi and DPGi inhibitory sites. These cells had an average firing frequency of $16.5 \pm 2.6 \text{ spikes s}^{-1}$ during muscle rigidity ($n = 8$, min = 7 spikes s^{-1} , max = 25 spikes s^{-1}).

KA microinjections into the Gi and DPGi

The firing rates of seven RMC and five RMC-spinal neurons were analysed during KA microinjections ($n = 12$) into Gi and DPGi inhibitory sites previously identified

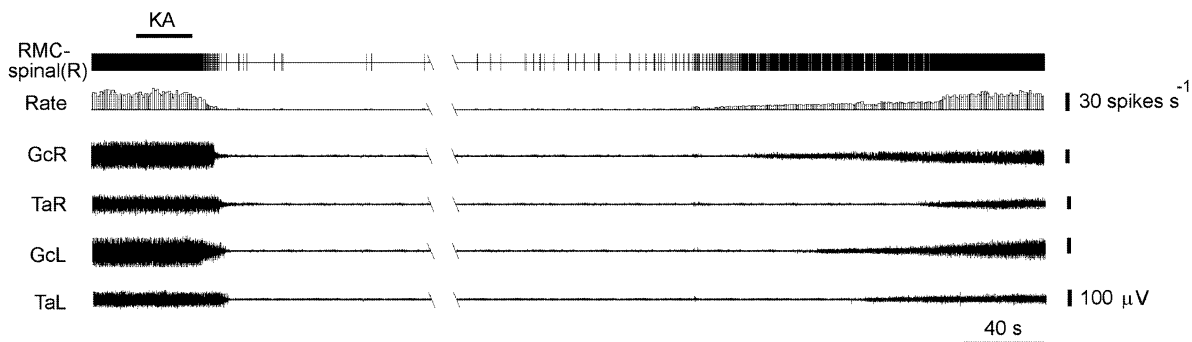


Figure 4. Inhibition of a RMC-spinal neuron after KA microinjection in the Gi site producing bilateral muscle atonia

The top horizontal left bar indicates the time and duration of the KA injection.

with electrical stimulation (Fig. 2). All microinjections produced a bilateral short-latency decrease of rigid hindlimb muscle tone and reduction of discharges in both RMC and RMC-spinal neurons (Fig. 4). The latency of muscle tone suppression after KA microinjections ranged from 18 to 56 s, with a mean of 35.8 ± 3.5 s ($n = 12$). The duration of muscle tone suppression ranged from 192 s to 615 s, with a mean of 407 ± 46 s. Three RMC and two RMC-spinal cells were inhibited completely, and six cells exhibited a $75 \pm 3\%$ reduction in firing rate after KA microinjections into Gi and DPGi inhibitory sites (min = 65%, max = 82%, $n = 6$, $P < 0.05$).

Animals with induced locomotion

RMC-spinal neurons. Twelve spontaneously active RMC-spinal neurons (Fig. 5) were recorded during baseline resting conditions, induced locomotion and motor inhibition evoked by Gi and DPGi stimulation. During the resting condition, RMC-spinal neurons discharged with an average frequency of 13 ± 2 spikes s^{-1} ($n = 12$, min = 5 spikes s^{-1} , max = 27 spikes s^{-1}). When animals started locomotion, 10 RMC-spinal neurons showed rhythmic activity related to contralateral hindlimb stepping. The maximal activity of nine RMC-spinal

neurons (66 ± 11 spikes s^{-1} , $n = 9$, min = 32 spikes s^{-1} , max = 125 spikes s^{-1}) correlated with swing phases and decreased in stance phases (3.6 ± 0.7 spikes s^{-1} , $n = 9$, min = 1 spike s^{-1} , max = 7 spikes s^{-1}). The firing rate of one RMC-spinal neuron increased (to about 45 spikes s^{-1}) during stance phases and two showed only a tonic increase of firing rate (20–30 spikes s^{-1}) during locomotion. Electrical stimulation of Gi and DPGi inhibitory sites (50–120 μA) inhibited stepping and decreased the activity of all RMC-spinal neurons (Fig. 6A). The firing rate of nine RMC-spinal neurons was completely inhibited and the discharge of three cells was reduced by 64–82% compared with the baseline during resting conditions. After Gi and DPGi stimulation, RMC-spinal neurons recovered their baseline firing rates and following of locomotion-related rhythmic activity.

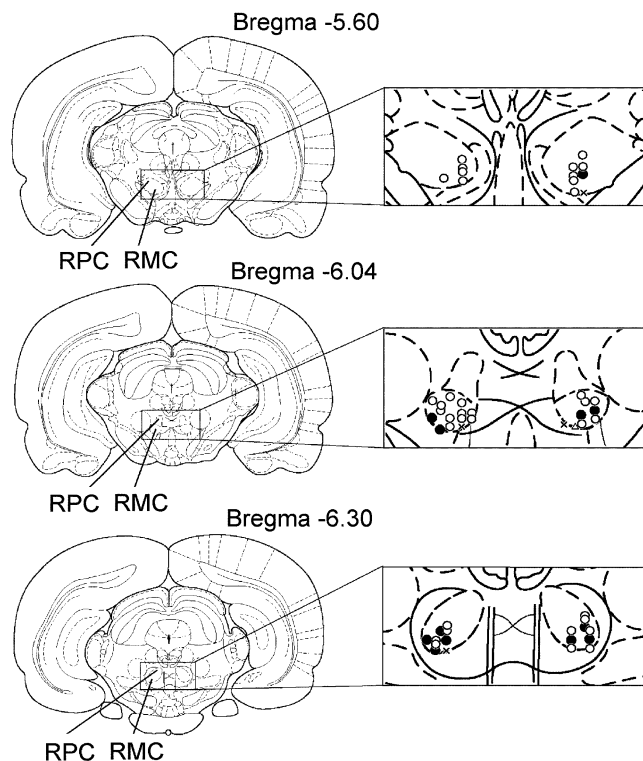


Figure 5. Coronal views of the rat midbrain showing the location of recorded RMC and RMC-spinal neurons

Open and black circles indicate RMC and RMC-spinal neurons, respectively, inhibited by Gi and DPGi stimulation, suppressing locomotion and the withdrawal reflex. Triangles indicate RMC neurons that were excited by Gi and DPGi stimulation. Crosses indicate RMC neurons that were unaffected by Gi and DPGi stimulation.

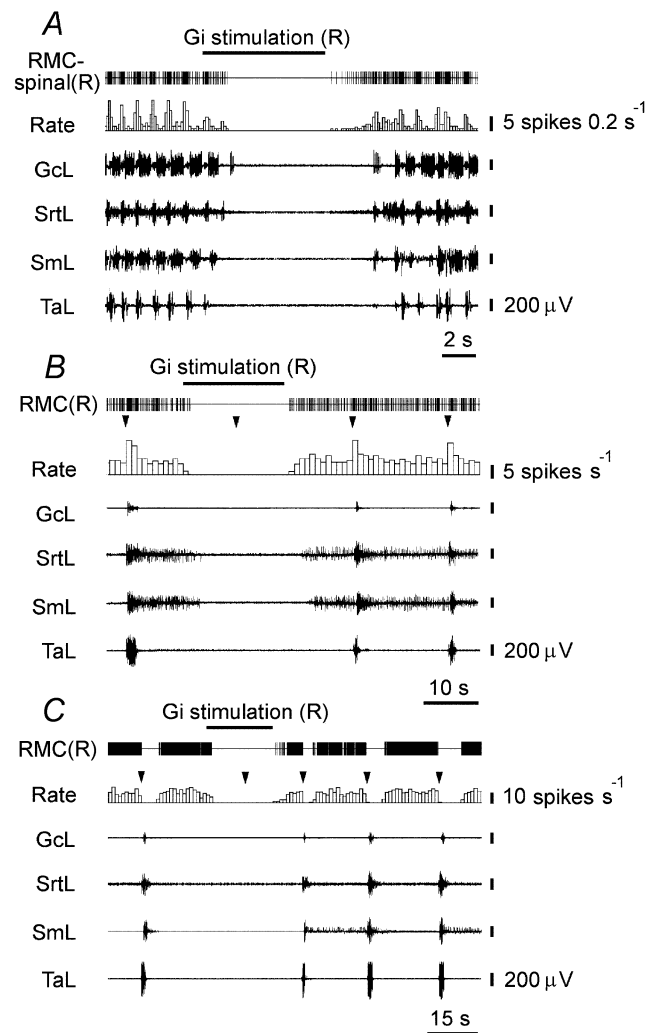


Figure 6. Inhibition of RMC-spinal and RMC neurons during Gi stimulation producing blockage of locomotion and the withdrawal reflex induced by hindlimb pinch

A, inhibition of the RMC-spinal neuron modulated by locomotor rhythm. B, C, inhibition of the RMC neurons suppressed and excited by a pinch; triangles indicate the time of pinch.

RMC neurons. The activity of 42 RMC neurons (Fig. 5) was additionally analysed during Gi and DPGi stimulation, which blocked the withdrawal hindlimb reflex evoked by contralateral hindlimb pinches. A total of 34 RMC neurons had a tonic discharge pattern with an average frequency of 8.4 ± 0.8 spikes s^{-1} ($n = 34$, min = 2 spikes s^{-1} , max = 22 spikes s^{-1}) and eight RMC cells showed spontaneous burst activity with an average burst duration of 4.1 ± 0.3 s ($n = 8$, min = 3 s, max = 5 s) and period between bursts 2.3 ± 0.3 s ($n = 8$, min = 2 s, max = 4 s) during the rest period. The maximal frequency of firing in bursts ranged from 8 to 48 spikes s^{-1} with a mean of 21 ± 4 spikes s^{-1} ($n = 8$).

All RMC neurons could be divided into one of three groups based on their responses. The first group ($n = 9$) consisted of RMC neurons whose firing rate increased by an average of 253 ± 33 % during the hindlimb withdrawal reflex compared with the baseline (min = 80 %, max = 367 %, $P < 0.01$). Neurons in the second group ($n = 14$) exhibited a firing rate increase (150 ± 20 %, min = 53 %, max = 275 %, $P < 0.01$; 0.8 ± 0.1 s, min = 0.3 s, max = 1.2 s) followed by complete inhibition for 3.8 ± 0.5 s (min = 1 s, max = 7 s). RMC cells in the third group ($n = 19$) were completely inhibited for 3.6 ± 0.5 s after the pinch was applied (min = 1 s, max = 8 s).

Gi and DPGi stimulation produced withdrawal reflex suppression and profoundly inhibited the activity of RMC neurons in all three groups. In the first group, four neurons were inhibited completely (Fig. 6B) and the firing rates of four neurons were decreased by 50–75 % compared with baseline. One RMC neuron in the first group did not respond to Gi stimulation. In the second group, six neurons ceased their discharge and eight neurons reduced their firing rate by an average of 72 ± 4 % (min = 55 %, max = 82 %, $P < 0.03$). In the third group, seven neurons were inhibited completely (Fig. 6C) and nine neurons exhibited a reduction in their discharge rate by an average of 74 ± 3 % (min = 60 %, max = 85 %, $P < 0.01$). Two RMC neurons in the third group did not respond to Gi stimulation and the firing rate of one neuron was increased by 100 %.

DISCUSSION

Our results show that electrical and chemical stimulation of Gi and DPGi sites that suppress muscle tone and locomotion reduced the activity of RMC and RMC-spinal neurons, which contribute to both the control of phasic parameters of movement and static torque (Mewes & Cheney, 1994). This reduction may be related to both partial disfacilitation (Tsukahara *et al.* 1965) and the direct inhibition of rubral cells (Ralston & Milroy, 1992). Disfacilitation in rubral neurons might be a result of inhibition of transmission in ascending somatosensory pathways during the excitation of brainstem inhibitory

regions (Kirzon & Kaplan 1978; Katayama *et al.* 1984) and a decrease in excitatory influences from external sources. Suppression of adrenergic and serotonergic systems during excitation of brainstem inhibitory sites (Wang *et al.* 1976; Taek, 1991; Mileykovskiy *et al.* 2000; Lai *et al.* 2001) or rapid eye movement (REM) sleep (Hobson *et al.* 1975; Fornal *et al.* 1985; Jacobs, 1986) might disfacilitate rubral neurons. However, microiontophoretic applications of serotonin and noradrenaline induce a significant level of depression in the majority of RN cells (Licata *et al.* 1998; Ciranna *et al.* 2000). Glutamate and acetylcholine also are not likely to be involved in the process of disfacilitation of rubral neurons since REM sleep is accompanied by increased release of these neurotransmitters (Kodama *et al.* 1992, 1998), which produce cell excitation in the RN (Marshall *et al.* 1980; Nieoullon *et al.* 1988; Kinney, 1995; Licata *et al.* 1998). On the other hand, direct inhibition of rubral neurons may be related to the activation of a local interneuron network in the RN, as well as distant inhibitory influences. Electron microscopy studies have demonstrated the presence of GABA immunoreactive cells that form symmetrical (inhibitory) synapses on RMC-spinal neurons, and GABAergic terminals from extranuclear sources (Ralston & Milroy, 1992). Intracellular recording has demonstrated that electrical stimulation of the dorsolateral mesencephalic reticular formation (DLMRF), including the deep mesencephalic nucleus, elicits monosynaptic hyperpolarizing postsynaptic potentials in about 95 % of rubral neurons. These hyperpolarizing potentials were blocked reversibly by bicuculline, a GABA_A receptor antagonist. Histochemical and electron microscopy studies have also demonstrated the existence of direct GABAergic projections from the DLMRF to RN neurons (Fu *et al.* 1996). Since neurons of the DLMRF receive excitatory inputs from medullary inhibitory sites and participate in the inhibition of muscle tone in anaesthetized and decerebrate animals (Sinnamon *et al.* 1987; Mileikovskii *et al.* 1991; Mileykovskiy *et al.* 2002), this midbrain region might be involved in the inhibition of rubral neurons during Gi stimulation. The Gi also sends projections to the pontine oral reticular nucleus (PnO), which is involved in the induction of REM sleep muscle atonia (Henley & Morrison, 1974; Shammah-Lagnado *et al.* 1987; Chase & Morales, 1990; Lai & Siegel, 1990; Siegel *et al.* 1992). Electrical stimulation of the PnO evokes GABA and glycine release in the vicinity of the locus coeruleus and inhibition of noradrenergic neurons in this nucleus (Mileykovskiy *et al.* 2000). The PnO, like the DLMRF, receives excitatory inputs from medullary inhibitory sites (Mileikovskii *et al.* 1991), therefore inhibition of RN cells during Gi stimulation might also result from excitation of this pontine nucleus.

It is worth noting that the reduction in RN neuronal activity during medullary stimulation and firing resumption in the poststimulation period preceded muscle tone

suppression and recovery in our study. This indicates that the alteration in RN firing was not a result of the change of afferent inflow from the muscle groups (Cheney *et al.* 1988; Mewes & Cheney, 1994; Van Kan & McCurdy, 2001).

Since brainstem transection at the pontomedullary junction as well as pontine lidocaine injections attenuate the muscle atonia induced by medullary stimulation (Siegel *et al.* 1983; Kohyama *et al.* 1998), we propose that the inhibition of rostral brainstem structures, in particular the midbrain locomotor region, locus coeruleus (Mileykovskiy *et al.* 2000), and RN, may contribute to muscle atonia. Electrical stimulation of the RN evokes EMG facilitatory responses in forelimb and hindlimb flexor muscles (Rho *et al.* 1999). About 70% of neurons located in the magnocellular part of the RN participate in the dynamic control of movement, and 27% of these cells have a tonic component of discharge, which contributes to static torque (Mewes & Cheney, 1994). These data suggest that cessation of the activity of RN cells during stimulation of the medial medulla reduces activity in circuits related to both the phasic and tonic regulation of muscle activity. Moreover, cessation of the activity in RN neurons may influence the integrative functions of the cerebellar nuclei participating in the regulation of postural muscle tone and motor control (Keifer & Houk, 1994; Keifer 1996; Pananceau *et al.* 1996; Jiang *et al.* 2002).

Terminals of corticorubral axons originating from the ipsilateral primary and supplementary motor cortex are mainly mapped in the parvocellular part of the RN, and only area 4 of the cortex sends a small direct projection to the RMC (Burman *et al.* 2000). Removing the motor cortex does not significantly change the cell discharges associated with finger movements in the denervated RMC (Houk *et al.* 1988). On the other hand, the RMC receives dense projections from the interpositus nucleus (Daniel *et al.* 1987, 1988). It was postulated that the cerebellorubral circuit, including the cerebellum, RN and reticular formation, acts as a positive loop that generates motor commands and conveys them to the spinal cord via the rubrospinal pathways (Orlovsky, 1972; Amassian & Batson, 1988; Arshavsky *et al.* 1988; Keifer & Houk, 1994). We propose that stimulation of the medial medulla may suppress transmission in this loop, which provides a dynamic cerebellar control of motor activity and regulation of the somatosensory information received from the dorsal column nuclei of the spinal cord (Fanardzhian & Sarkisian, 1984; Taepavaraprak *et al.* 2002).

The Gi and PnO are important components of the brainstem-reticulospinal inhibitory system, which participates in REM sleep atonia (Takakusaki *et al.* 1989; 1994; Chase & Morales, 1990; Morales *et al.* 1999; Hajnik *et al.* 2000). We propose that PnO and Gi cell groups, when activated during REM sleep, evoke the suppression of activity in excitatory brainstem motor systems in addition to

descending inhibition of spinal motoneurons, and this coordinated activity underlies muscle atonia during REM sleep and motor regulation in waking. RN unit recording across the sleep-waking cycle revealed that these cells decreased their activity in transition from waking to non-REM sleep and during tonic periods of REM sleep (Gassel *et al.* 1965; Harper & Jacobs, 1972). During the phasic events of REM sleep, the RN unit firing rate is significantly increased compared with quiet waking and other sleep stages. On the other hand, Jacobs *et al.* (1970) reported that some RN cells had higher firing rates in tonic periods of REM than in non-REM sleep. Unfortunately, recorded RN cells were not identified as rubrospinal neurons, therefore they might be elements of the GABAergic apparatus (Vuillon-Cacciuttolo *et al.* 1984; Ralston & Milroy, 1992) activated during REM sleep (Nitz & Siegel, 1997a, b). Alternatively, inhibitory influences from brainstem inhibitory sites might be partially masked by excitatory flows from structures of ascending activating reticular systems excited during REM sleep (Siegel *et al.* 1977; Shiromani *et al.* 1988; Cornwall *et al.* 1990; Steriade *et al.* 1990; Quattrochi *et al.* 1998). An increase in RN unit firing rate during REM sleep after lesioning of the brainstem inhibitory sites might demonstrate these inhibitory influences.

It is suggested that the reduction in the activity of mesopontine neurons and the reduction in eye movements during cataplexy compared with both REM sleep and wakefulness is related to active inhibition of the central motor systems during this state in narcoleptic dogs (Siegel *et al.* 1992). This hypothesis is supported by data showing that cataplexy is accompanied by excitation of a specialized subpopulation of cells in the ventromedial medulla (Siegel *et al.* 1991), a region that is responsible for REM sleep atonia (Sakai 1980; Lai & Siegel, 1988; Schenkel & Siegel, 1989). Moreover, cessation of the activity of locus coeruleus neurons in cataplexy (Wu *et al.* 1999) and during the electrical stimulation of medullary inhibitory sites (Mileikovskiy *et al.* 2000) also demonstrates the active nature of muscle tone suppression during cataplectic attacks. Thus, we propose that the activity of RMC-spinal neurons, like activity of locus coeruleus and mesopontine reticular cells, might be reduced in cataplexy, and this reduction is related to activation of the medullary neurons responsible for muscle atonia.

The current results together with our recent published data suggest that excitation of the pontine and medullary inhibitory regions evokes a generalized suppression of activity in brainstem facilitatory motor systems in addition to direct inhibition of spinal motoneurons. Moreover, brainstem inhibitory structures may block the descending excitatory influences addressed to spinal motor centres from rostral motor structures, and thus promote the spinal motoneuronal inhibition evoked by the reticulospinal

inhibitory system (Mileyskiy *et al.* 2000). Abnormal function of these brainstem inhibitory mechanisms or disruption of their coordinated activity may contribute to human motor disorders such as cataplexy, restless legs syndrome, dystonia and spastic cerebral palsy.

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