Discharge Characteristics of Neurons in the Red Nucleus During Voluntary Gait Modifications: A Comparison with the Motor Cortex

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Lavoie, Sylvain, and Trevor Drew. Discharge characteristics of neurons in the red nucleus during voluntary gait modifications: a comparison with the motor cortex. J Neurophysiol 88: 1791–1814, 2002; 10.1152/jn.00009.2002. We have examined the contribution of the red nucleus to the control of locomotion in the cat. Neuronal activity was recorded from 157 rubral neurons, including identified rubrospinal neurons, in three cats trained to walk on a treadmill and to step over obstacles attached to the moving belt. Of 72 neurons with a receptive field confined to the contralateral forelimb, 66 were phasically active during unobstructed locomotion. The maximal activity of the majority of neurons (59/66) was centered around the swing phase of locomotion. Slightly more than half of the neurons (36/66) were phasically active during both swing and stance. In addition, some rubral neurons (14/66) showed multiple periods of phasic activity within the swing phase of the locomotor cycle. Periods of phasic discharge temporally coincident with the swing phase of the ipsilateral limb were observed in 766 neurons. During voluntary gait modifications, most forelimb-related neurons (70/72) showed a significant increase in their discharge activity when the contralateral limb was the first to step over the obstacle (lead condition). Maximal activity in nearly all cells (63/70) was observed during the swing phase, and 2363 rubral neurons exhibited multiple increases of activity during the modified swing phase. A number of cells (18/70) showed multiple periods of increased activity during swing and stance. Many of the neurons (35/63, 56%) showed an increase in activity at the end of the swing phase; this period of activity was temporally coincident with the period of activity in wrist dorsiflexors, such as the extensor digitorum communis. A smaller proportion of neurons with receptive fields restricted to the hindlimbs showed similar characteristics to those observed in the population of forelimb-related neurons. The overall characteristics of these rubral neurons are similar to those that we obtained previously from pyramidal tract neurons recorded from the motor cortex during an identical task. However, in contrast to the results obtained in the rubral neurons, most motor cortical neurons showed only one period of increased activity during the step cycle. We suggest that both structures contribute to the modifications of the pattern of EMG activity that are required to produce the change in limb trajectory needed to step over an obstacle. However, the results suggest an additional role for the red nucleus in regulating intra- and interlimb coordination.

INTRODUCTION

Single-unit recording studies in cats (Almaric et al. 1983; Batson and Amassian 1986; Burton and Onoda 1978; Ghez and Kubota 1977; Houk et al. 1987; Martin and Ghez 1988, 1991; Padell and Steinberg 1978; Schmied et al. 1988), rats (Jarrett and Hyland 1999), and primates (Gibson et al. 1985a,b; Mewes and Cheney 1994; Miller and Houk 1995; Miller et al. 1993; Otero 1976; van Kan and McCurdy 2001) have shown that neurons in the magnocellular region of the red nucleus, including rubromotoneuronal cells (Fetz et al. 1989; Mewes and Cheney 1994), increase their discharge frequency during the execution of voluntary movements. Detailed analysis of these discharge patterns suggests that in most cases the increase of discharge frequency precedes the onset of the movement (Almaric et al. 1983; Ghez and Kubota 1977; Gibson et al. 1985b; Martin and Ghez 1988; Mewes and Cheney 1994; Otero 1976) and may be correlated with different parameters of the movement, such as the velocity (Burton and Onoda 1978; Gibson et al. 1985b; Mewes and Cheney 1994), or with the temporal characteristics of the electromyographic (EMG) activity that produces that movement (Miller and Houk 1995; Miller and Sinkjaer 1998; Miller et al. 1993). Furthermore, the studies of several authors (Gibson et al. 1985a, 1994, 1998; Mewes and Cheney 1994; Miller et al. 1993; Sinkjaer et al. 1995; van Kan and McCurdy 2001) suggest that the red nucleus may play a particular role during coordinated, multi-articular movements such as reaching, particularly when these movements involve the use of the hand e.g., reach-to-grasp.

During locomotion, the only information on the discharge patterns of rubral neurons comes from experiments in the decerebrate cat (Arshavsky et al. 1988; Orlovsky 1972a). The results from these experiments show that rubrospinal neurons are phasically active during locomotion in the decerebrate cat and discharge preferentially in the swing phase of locomotion where they could influence the activity of flexor muscles (Orlovsky 1972a). This is in agreement with the results from studies in both the decerebrate (Degtyarenko et al. 1993; Orlovsky 1972b) and intact cat (Rho et al. 1999) showing that microstimulation within the red nucleus during locomotion preferentially modifies the activity of physiological flexor muscles. Nevertheless, both the unit recording and microstimulation studies indicate that the red nucleus may also influence the activity of extensor muscles active at the end of the swing phase and during stance. Electrolytic lesion (Ingram and Ranson 1932), excitotoxic lesion (Muir and Wishaw 2000), or pharmacological inactivation (Gibson et al. 1994) of the red nucleus leads to evident but relatively mild locomotor deficits during overground locomotion, although the study of Ingram...
and Ranson (1932) reported stronger deficits when the cats walked in a cluttered environment. Taken together, the data suggest that the red nucleus does contribute to the normal control of walking, although the nature of this contribution is unclear, in part because of the lack of any data on cell discharge characteristics in the intact, walking cat. Moreover, given the comments of Ingram and Ranson (1932) concerning the increased loss of control in more challenging circumstances and the wealth of evidence in primates demonstrating the importance of the red nucleus in the control of voluntary movements, it seems probable that the red nucleus, like the motor cortex (Amos et al. 1990; Armstrong 1988; Beloozerova and Sirota 1993; Drew 1988, 1993; Drew et al. 1996; Widajewicz et al. 1994), might contribute more strongly to the regulation of locomotion when the cat has to adapt its gait to the environment. To test this general hypothesis, we used single-unit recording techniques in the intact cat to characterize the nature of the discharge characteristics of rubral neurons during a task requiring a modification of the base locomotor rhythm. To allow direct comparison of the results from this study with those that we have previously obtained from our recordings of identified pyramidal tract neurons (PTNs) in the motor cortex (Drew 1993; Widajewicz et al. 1994), we used the identical task requiring the cats to modify their gait to step over an obstacle attached to a moving treadmill belt. This task requires that the cats adjust the spatiotemporal pattern of the muscle activity in their limbs to produce the changes in limb trajectory needed to step over the obstacle without touching it. As such, it provides an appropriate method for characterizing the discharge characteristics of rubral neurons in a situation in which the locomotor pattern has to be modified.

A preliminary report of this study has been published as an abstract (Lavoie and Drew 1997).

METHODS

Training and surgery

Experiments were carried out on three cats (weight: 5.4–5.9 kg) that were initially trained to walk on a treadmill at speeds circa 0.4 m·s⁻¹ and to step over obstacles attached to the moving belt (see Drew 1988, 1993). These were the same three animals that were used in our previously published paper detailing the effects of microstimulation of the red nucleus on the locomotor rhythm and pattern (Rho et al. 1999). Following training, the cats were prepared for surgery under general anesthesia and in aseptic conditions. All procedures followed the recommendations of the Canadian Council for the Protection of Animals and were approved by the local animal ethics committee at the Université de Montréal.

The surgical procedures used in these experiments are detailed in Rho et al. (1999). In brief, a recording chamber was attached over a craniotomy in the parietal bone to provide access to the red nucleus on the right hand side. A bundle of three microwires was implanted stereotaxically into the left brachium conjunctivum at P-7.5, V-3, L3.8, and, in two animals, a similar bundle was implanted stereotaxically into the left rubralspinal tract at P9.2, V-8.5, and L4.5. In two animals, three microwire electrodes were inserted manually into the left dorsolateral funiculus at L₇. In all animals, pairs of Teflon-insulated, braided stainless-steel wires were implanted into selected muscles of all four limbs to record EMG activity during locomotion. The selected muscles included physiological flexor and extensor muscles acting around all the major joints of the forelimb and hindlimb contralateral to the recording site (see Rho et al. 1999 for a list of these muscles and their major functions). Each animal was administered Buprenorphine (hydrochloride, 5 µg/kg) for the 48 h following the surgery. Antibiotics (cephalexin monohydrate 50 mg/kg) were administered throughout the period of study of these animals. Experiments were started 1 wk after the surgery.

Protocol

Glass-insulated, tungsten microelectrodes (impedance: 0.5–2.0 MΩ), held in a custom-made micromanipulator attached to the recording chamber, were driven through the parietal cortex and the superior colliculus to a position previously calculated to be just dorsal to the red nucleus. The electrode was then advanced slowly into the red nucleus. In initial experiments, the exact location of the forelimb representation of the red nucleus was determined on the basis of the presence of a large, short-latency (<1 ms) field potential following stimulation of the electrodes in the brachium conjunctivum (see e.g., Figs. 2C and 5C), of large neurons with a cutaneous receptive field on the contralateral forelimb and by brief, twitch responses, restricted to the forelimb, following stimulation through the microelectrode (11 pulses at 330 Hz, pulse duration: 0.2 ms) at strengths of <20 µA (not illustrated). In these initial experiments, the electrode was then withdrawn ~1 mm and left in place for 10–15 min before being again slowly advanced into the red nucleus. The data from these initial experiments were used to directly position the electrode above the predicted location of the red nucleus in later experiments.

As the electrode was advanced, action potentials that were clear of the noise were tested for a receptive field by gentle manipulation of the body. In many penetrations, the action potentials that were initially isolated had receptive fields around the face or neck of the animal (see Fig. 1). These cells were not routinely recorded during locomotion, although a number of such cells, in all three cats, were sampled to determine whether they were phasically active during the task. Below these cells, or intermingled with them, we isolated cells with receptive fields that were restricted to or included the contralateral forelimb. From this point on, the discharge frequency of all neurons with stable, isolated action potentials were recorded during locomotion with either one or two obstacles attached to the treadmill belt. Unit and EMG activity were recorded on a 14-channel instrumentation tape recorder, and we also made simultaneous video recordings of the cat during this time. A digital time code simultaneously recorded on both media allowed synchronization of these recordings. Following the recordings during locomotion, the cat was removed from the treadmill, and the receptive field of the cell was more carefully mapped by gentle manipulation of the limbs and body of the animal. In sites at which well-modulated activity was recorded during locomotion, short trains of microstimulation at 25 µA were applied and the evoked movements, as well as the threshold for the effects noted (see Rho et al. 1999). The cat was then replaced in the treadmill and the electrode advanced until another single unit was isolated. Recording sessions normally lasted for 2–3 h and terminated when the cats were no longer willing to walk. In selected penetrations, small electrolytic lesions were made above, below or, occasionally, within the predicted borders of the red nucleus to aid in histological reconstruction of the electrode penetrations.

Following all of the recording and stimulation procedures, the same cats were used to examine the effects of microinjections of muscimol into the red nucleus (not illustrated in this report).

Data analysis

Following the experiments, the data were displayed on an electrostatic printer (Gould ES 2000), and sections of data in which the action potential and the locomotion were stable were selected for analysis. Action potentials were amplitude-discriminated and transformed into digital pulses; these were sampled, together with the
EMG data, at a frequency of 1 kHz. A custom-written program was used to mark the onset and offset of the periods of EMG activity and to identify the step cycles during which the cat stepped over the obstacle as well as those cycles preceding this step. As in our previous studies (Drew 1993; Widijawicz et al. 1994), steps over the obstacle were further divided into those in which the leg contralateral to the recording site was the first to pass over the obstacle (the lead limb) and those when it was the second (trail limb). The identification of these steps was based on inspection of the simultaneously recorded videos; the latter were also used to ensure that the cat neither touched the obstacle nor hesitated during the gait modification. Control cycles were defined as those that occurred two steps before the one over the obstacle as our previous studies showed that neither EMG activity nor the discharge pattern of PTNs was different during this step than

**FIG. 1.** Location of representative examples of electrode penetrations in cat RN4. A: digitized image of a photomicrograph of the red nucleus at a laterality (L) of 2.4 mm from the midline showing the location of penetrations 32 and 15. A 3rd penetration can be seen between the 2 that are identified on the figure. The figurines to the right of the photomicrograph illustrate the receptive fields of cells recorded in each of the identified penetrations. The numbers beside each of the figurines indicate the calculated location of each of the recorded cells as indicated on the photomicrograph. B and C: organized in the same manner as in A but show tracings of the histological sections instead of photomicrographs. Shaded regions on the figurines indicate cutaneous receptive fields, curved arrows indicate that the cell was activated by movement of the indicated part; the pair of small straight arrows in cell 6 in track 11 indicate that this cell was activated by pressure applied to the paw. FL, the receptive field was identified as being on the forelimb but could not be more precisely defined because the cell was lost before identification was complete; 3N, oculomotor nerve; PG, pontine gray; PT, pyramidal tract; RN, red nucleus; SN, substantia nigra; T, track (or penetration); TRN, tegmental reticular nucleus.
during locomotion when no obstacles were attached to the treadmill belt (Drew 1993).

Once identified, the selected cycles were used to construct averages that were triggered on the onset of either the contralateral cleidobracialis (coClB) or the contralateral sartorius (coSrt), the onset and duration of which correspond, approximately, to the onset and duration of the forelimb and hindlimb swing period, respectively (Drew 1993; Widajewicz et al. 1994). To average the signals, both the instantaneous discharge frequency of the cell and the EMG activity during each cycle was divided into 256 bins (Drew 1993; Drew and Doucet 1991; Udo et al. 1982). The discharge activity during the steps over the obstacle was superimposed on the activity during the control cycle. The latter activity was displayed together with the 0.01 confidence interval of the standard error of the mean. Periods of activity that exceeded the upper or lower boundaries of this level of confidence for more than 25 consecutive bins were deemed to be significantly different from control activity (Drew 1993). When two obstacles were attached to the treadmill, the data from each were combined. Unit activity was also displayed in the form of rasters, triggered successively on the onset of each of the recorded muscles, to examine the timing relationships between the periods of unit activity and the period of activity of each of the recorded muscles (Drew 1993; Drew et al. 1986).

To determine whether cells were phasically modulated and during which parts of the step cycle they showed peak activity, we used a combination of methods. First, we used circular statistics and the Rayleigh test for directionality (P < 0.01) to determine which cells showed phasic (i.e., nonuniform discharge) modulation (Batschelet 1981; Drew 1993; Drew and Doucet 1991). However, because many of the cells in the red nucleus showed multiple periods of discharge activity (see RESULTS), we could not use circular statistics to routinely determine the mean phase and level of activity of these cells in the same way as for PTNs (Drew 1993). For those cells that were phasically modulated, we therefore determined the phase of neuronal discharge from normalized averages of the cell discharge, triggered on the onset of coClB or coSrt. For control steps, the overall average level of the discharge during each step cycle was calculated, and the points at which the histogram crossed this level were considered to represent the onset and offset of the period of burst activity (see e.g., Figs. 2B and Fig. 3). To quantify the data during the steps over the obstacle, similar methods were used except that we found that for four cells, some significant increases in discharge were too small to be included on the basis of the overall average. For these four cases, the peak of discharge was calculated on the basis of the overall discharge rate for the major burst as well as on the basis of mean discharge for the minor, but significant, burst; an example of one of these four cells can be seen in Fig. 6A. It should be noted that a comparison of the phase of activity of the cortical neurons obtained using the current method with that obtained using circular statistics showed only small differences in the values obtained using the two methods (compare Fig. 4D in this report with Fig. 5 in Drew 1993).

To identify the part of the step cycle during which cells were maximally active during the gait modifications, they were classified in a similar manner to our previous publications. Three of these groups were identical to those previously used to identify PTNs. For cells related to the forelimbs (see RESULTS), these were cells whose peak discharge activity occurred just prior to, or just after, the onset of the activity in the coClB but prior to the onset of activity in the wrist and digit dorsiflexor, coEDC (phase I cells); cells whose peak discharge occurred subsequent to the onset of activity in the EDC but before the end of the period of activity in the coClB (phase II cells); and cells in which the increase in discharge began >200 ms prior to the onset of activity in the coClB (early). Because we identified a group of cells in the red nucleus whose activity was temporally related to the swing period of the ipsilateral limb (see e.g., Fig. 3A), and thus discharged during the stance phase of the contralateral limb, we subdivided our previous stance classification (Drew 1993) into cells whose peak discharge was included within the period of activity of the iCIB (i.e., ipsilateral swing cells); and those cells whose peak discharge occurred outside the period of the coClB activity, excluding group 4 (i.e., stance cells). In addition, on the basis of our initial inspection of the discharge patterns of these cells, we designated a further two categories: cells whose discharge activity was maximal throughout the period of swing (phase I + II cells) and those cells that showed a period of activity in phase I and another in phase II (phase I and phase II cells). For cells related to the hindlimbs, the same classifications were maintained with respect to the coSrt and the extensor digitorum brevis (EDB).

**Results**

**Database**

A total of 157 neurons from 45 penetrations were recorded during locomotion. All of these penetrations were histologically verified to lie within the boundaries of the red nucleus with most trajectories lying within the caudal two-thirds of the nucleus that is normally defined as the magnocellular region of the red nucleus (Massion 1967; Padel et al. 1972; Pompeiano and Brodal 1957; Robinson et al. 1987).

Figure 1 illustrates histology from one cat, RN4, and shows the location of some of the penetrations made at different lateralities in this cat, together with the receptive fields of the cells recorded in representative penetrations. In accord with previously published reports, we found that most cells with receptive fields confined to the contralateral hindlimb were located in the more lateral and ventral regions of the red nucleus (see e.g., tracks 15 and 32 in Fig. 1A), whereas neurons with receptive fields restricted to the forelimbs were more scattered throughout the red nucleus but were concentrated in the more medial and dorsal regions (tracks 11, 24, 25, and 29 in Fig. 1, B and C). Cells with receptive fields confined to the face, neck and proximal forelimb were normally found in the most dorsal parts of the trajectories, especially in more rostral regions (e.g., tracks 11 and 29), although cells with very distal receptive fields were also sometimes recorded in these most dorsal regions (e.g., cell 1 in track 11).

Receptive fields were tested for 138/157 of these neurons and could be identified for 129/138. Of these, the majority (72/129, 56%) had a receptive field that was restricted to the
contralateral forelimb, and, for most of these (50/72) the receptive field was restricted to those parts of the forelimb including and distal to the elbow. A further 22/129 (17%) had a receptive field that was restricted to the hindlimbs. In part, this preponderance of cells with receptive fields on the forelimb, compared with the hindlimb, is a result of a bias in the recordings in that the cat was normally unwilling to continue walking by the time that the penetration had advanced to the more ventral regions of the nucleus where the hindlimb cells were concentrated. Relatively few cells were recorded with receptive fields restricted to the face and neck (15/129, 12%) or the trunk (4/129, 3%), largely because of our tendency to advance the electrode rapidly through the most dorsal regions of the red nucleus where these cells were most frequently located. Of the remaining cells, 14/129 (11%) had a receptive field that overlapped more than one of the above categories and 2 cells had a receptive field restricted to the ipsilateral forelimb. The vast majority (120/129) of these neurons were activated by brushing or light tapping of the skin; because of their extreme sensitivity to these cutaneous stimuli, we were unable to accurately determine if they received additional deeper or proprioceptive afferent input. The other neurons (9/129) were activated only by deep tapping or joint movement (see e.g., track 11 in Fig. 1C).

The electrodes implanted into the brachium conjunctivum were accurately positioned in two of the cats (RN3 and RN5). Stimulation through these electrodes orthodromically activated a substantial proportion of the cells (66/93, 71%) that we recorded in these two cats. Unidentified cells were often recorded interspersed among the identified cells, suggesting that the lack of identification was probably due to an inability to completely activate the brachium (see Eccles et al. 1975b). The electrodes directed at the rubrospinal tract in the brain stem were accurately positioned in only one of the two cats in which they were inserted. These electrodes antidromically activated 19/42 (45%) of the tested cells in this cat. The actual percentage was probably higher but the very-short-latency responses were masked by the stimulus artifact. Spinal cord electrodes were accurately positioned in one of the two cats implanted. In this animal, 4/5 of the rubral neurons with a receptive field on the hindlimb were antidromically identified from these electrodes; in this same cat, none of the 16 neurons with a forelimb receptive field were activated from these electrodes.

Cell discharge during locomotion

Although the major objective of this study was to examine the discharge characteristics of rubral neurons during voluntary gait modifications, we first present data on the background discharge characteristics during normal locomotion in the intact cat. This is necessary as no other detailed source of information is available for locomotion in the intact cat and it also allows comparison with the only other detailed study available which was obtained in the decerebrate cat (Orlovsky 1972a). Because of the nature of our task, in which the forelimbs are obviously the first to encounter the obstacle, we concentrate on the 72 cells in which the receptive field was located exclusively on the forelimb. Data for the hindlimb, face, and neck are presented more concisely at the end of RESULTS.

CELLS WITH A RECEPTIVE FIELD RESTRICTED TO THE FORELIMB. Discharge activity during control locomotion. Of the 72 neurons with receptive fields restricted to the forelimbs, 66 discharged phasically as determined by the Rayleigh test for directionality. Figure 2 shows the discharge characteristics of one phasically active rubral neuron in the control cycles between gait modifications. Because only one obstacle was attached to the treadmill belt during this recording, there were seven or eight steps between the steps over the obstacle. This rubral neuron had a receptive field that extended from the paw to the upper arm but that was most intense on the dorsum and ventral surface of the paw: the neuron was orthodromically activated from the electrodes in the brachium conjunctivum at a latency of 1.2 ms (Fig. 2C). During locomotion, the neuron discharged in a clearly rhythmic manner with one intense peak of activity at the end of the swing phase, coincident with the period of activity of the wrist and digit dorsiflexor, EDC (Fig. 2, B and D). However, as in most of the rubral neurons that we recorded, the cell did not discharge in a simple unimodal pattern but showed a more complex pattern of peaks and troughs with another prominent peak of discharge activity at the end of stance, just prior to foot lift.

Two other examples of the discharge characteristics of cells with receptive fields on the forelimb are illustrated in Fig. 3. As for the example in Fig. 2, both of these neurons exhibited more than one period of phasic activity during control locomotion. The first of these examples (Fig. 3A) discharged with one burst of activity during the swing phase of the contralateral forelimb and the other burst during the following stance phase. Given that this burst was discrete and aligned well with the period of activity of the iClB (bottom), there is the strong possibility that this second burst of activity was related to the swing phase of the ipsilateral forelimb (see DISCUSSION). The other cell (Fig. 3B) also showed a complex pattern of activity, discharging most intensely at the time of paw lift but also discharging in quite discrete bursts of activity at the end of swing (during the period of activity of the coEDC) and again during stance. In this example, this latter period of activity did not align with the period of activity of the iClB.

Figure 4 plots the period of activity of each of the 66/72 phasically modulated forelimb neurons as a function of the phase of the step cycle when they were active (see METHODS). The majority of the neurons (46/66, 70%) exhibited multiple periods of activity during locomotion and showed at least some phasic activity during both swing and stance (36/66). Fourteen (14/66) neurons showed more than one phasic period of activity during the swing phase. Figure 4B (□) shows that the maximal period of activity of the majority of cells (59/66) was centered around the swing phase of locomotion, extending from the end of stance (phase 0.9) to the time of paw contact (phase 0.4). The second small peak that is evident in this plot corresponds, approximately, to the period of swing of the ipsilateral forelimb. The open bars in Figure 4C (see legend) indicate that there was a substantial level of lower frequency phasic activity during the stance phase of locomotion (0.4–0.9).

These data are compared in Fig. 4, D and E, with the discharge characteristics of 73 phasically active motor cortical cells (from a total of 91 neurons) recorded in a previous study (Drew 1993). All of these 73 cells were histologically confirmed to have been recorded from area 4, they were all identified as projection neurons (PTNs), and they were all
recorded within the forelimb representation of the motor cortex. As in the present study, we recorded all isolated cells so that there was no preselection on the basis of discharge characteristics. Inspection of the data in Fig. 4 shows several differences between the rubral and cortical populations. In particular, fewer of the motor cortical cells (14/73, 19%) showed more than one phasic period of activity during the step cycle, and only four PTNs exhibited more than one period of activity that overlapped the swing phase. In addition, the periods of discharge activity were more uniformly dispersed throughout the step cycle, with a relatively high proportion of cells showing maximal peak activity during stance (Fig. 4E). There were also clear differences in the frequency of the discharge in red nucleus and motor cortical cells. As illustrated in Fig. 4, C and F, whereas red nucleus cells frequently discharged at >50 Hz (52/66, 79%) and sometimes at >100 Hz.
FIG. 3. Examples of 2 other rubral neurons during control cycles. The data are arranged in the same manner as for Fig. 2, i, ipsilateral.
Hz (28/66, 42%), only 20/73 (27%) motor cortical cells discharged at >50 Hz and none of them surpassed 100 Hz (see also Armstrong and Drew 1984).

Discharge activity during gait modifications: lead condition. We have already detailed the major kinematic, kinetic, and EMG changes that occur when the cats modify their gait (Drew 1993; Kably and Drew 1998; Lavoie et al. 1995; Prentice and Drew 2001; Widajewicz et al. 1994), and they will, therefore, be only summarized here. In brief, the lead forelimb is brought above and over the obstacle by the coordinated and sequential modification of the activation patterns of physiological flexor muscles acting around all of the major joints of the limb. The

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**FIG. 4.** Distribution of the periods of discharge activity of rubral and cortical cells during control locomotion. A: periods of activity for 66 phasically modulated rubral neurons. Each horizontal line indicates one period of activity as defined in METHODS. ●, the time of peak activity in each cell. The cells are rank-ordered according to the phase of onset of the earliest period of activity. The cells illustrated in Figs. 2 and 3 are identified to the left side of the figure. B: phase of maximal peak activity of the 66 rubral neurons (●). ●, the phase of peak discharge for all periods of phasic activity (that is 1 value for each - - - in A). C: distribution of the maximal discharge frequency of each rubral neuron. D–F: similar displays for 73 phasically active pyramidal tract neurons (PTNs) recorded from the motor cortex.
modifications of the EMG pattern include changes in the duration (e.g., CIB), the amplitude (e.g., Br), and the relative timing of muscle activity (e.g., EDC) (see Figs. 5B and 11). During the subsequent extension of the limb as the animal prepares for contact with the treadmill surface, there is an increased activation of the elbow extensors as well as the wrist dorsiflexor muscles; the latter presumably stabilize the paw prior to it being replaced on the treadmill surface. There are also changes in the extensor muscles during the subsequent stance phase that provide postural support as the other limbs step over the obstacle.

The discharge activity of most rubral neurons (70/72) with a

![Graphical representation of neuronal activity and EMG patterns during gait modification.](image-url)
receptive field restricted to the forelimb was modified during the steps over the obstacles. In all of these cells, the modification occurred as the limb contralateral to the recording site stepped over the obstacle, and there was no modification in activity when the contralateral hindlimb stepped over. As in the example illustrated in Fig. 5, in many cases, this modification took the form of a substantial increase in the discharge frequency during the swing phase of the step over the obstacle. In the illustrated example, the discharge frequency increased from circa 55 Hz during the control cycles to circa 140 Hz during the step over the obstacle. There was also a slight change in the period of maximal activity of this cell that started to increase its discharge before the onset of the coCiB and coBr activity during the control step but that was much more tightly related to the period of activity of the coBr during the step over the obstacle (see Fig. 5, B and D).

While several cells showed a simple increase in activity similar to that illustrated in Fig. 5, the majority of rubral neurons showed multiple increases in activity during the modified step, as shown in Fig. 6. Figure 6A illustrates an example of a cell that discharged intensely throughout swing (classified as phase I + phase II) and, in addition, also exhibited a discrete and significant increase in its discharge frequency during the following stance phase. The timing of this burst of activity corresponded to the period of activity in the iCiB as the ipsilateral (trail) forelimb stepped over the obstacle.

The other three illustrated cells also showed more than one peak of activity during swing. Figure 6B, C and D, shows two cells, from two different cats, that showed increased activity both in early and late swing (phases I and II), with the second burst of activity aligning well with the onset and offset of the coEDC. In neither of these examples did the cell discharge in phase I begin with, or continue throughout, the period of activity of the coCiB. In addition, while the initial peak of activity, during phase I, in the cell illustrated in Fig. 6B represented an increase over that observed during unobstructed locomotion, the analogous peak in the cell illustrated in Fig. 6C represents a new period of activity, at a time when the cell was inactive during the control locomotion. The cell in Fig. 6B (which is the same as that illustrated during control locomotion in Fig. 3A) also showed a further change in activity during the stance phase. This involved a change in phase rather than an increase in the level of activity and was the result of a delay in the onset of swing in the trail limb during the step over the obstacle, compared with the control situation. The final example illustrated (Fig. 6D) also showed multiple peaks of activity during the swing phase of locomotion, but in this case, there was an increase in the discharge frequency only of the period of activity in phase II. There was also an increase in the discharge activity during the ipsilateral swing phase.

The relative frequency of the different types of changes are shown in Table 1, which allocates cells to categories using the definitions given in the Methods. The majority of cells (66/70) were maximally active during the swing phase, and in 63/66 neurons, this represented an increase in discharge activity over control. Of these, 12/63 showed a single increase in activity during early swing (phase I) and a further 12 only during late swing (phase II). The former group was not homogeneous and included cells whose discharge temporally correlated best with the teres major (TrM) and others that peaked later and correlated best with the period of activity in the wrist dorsiflexors, such as ECR (see Fig. 11). The latter group (phase II) was more homogeneous, and the discharge in these cells all coincided with the period of activity in the EDC that just preceded contact of the paw with the treadmill belt. A further 16 rubral neurons showed a maintained increase in discharge throughout the swing period, and 23 neurons showed significant increases in activity during both phases I and II. For these latter neurons, the phase of the initial peak showed the same variability as those cells that showed an increased discharge only during phase I, and the second peak showed the same homogeneity of phase as those cells that discharged only during phase II. In many cases, the peaks of significantly increased activity during the steps over the obstacle were generated at phases of the step cycle at which no peaks were detected during the unobstructed locomotion.

Some cells (22/70) also showed increased activity during the stance phase of the step cycle following the passage of the lead limb over the obstacle; all except 4/22 of these cells also showed increased activity during swing. For 13/22 of these neurons, the increase in discharge activity was relatively discrete and overlapped with the swing phase of the ipsilateral limb as it passed over the obstacle. Examples of this type of activity can be seen in Fig. 6, A, B, and D. All 13 neurons that showed this type of discharge displayed at very similar times in the step cycle with the phase of peak activity ranging from 0.66 to 0.80 of the cycle (mean = 0.72 ± 0.04; mean ± SD). Note that a further six rubral cells discharged phasically during the swing phase of the ipsilateral limb but did not show significant activity. The remaining 9/22 neurons that showed increased activity during stance either showed a longer period of increased activity that was temporally related to the period of activity of the coTrIL muscle (6/8) or discharged more discretely at the end of the stance phase. Eight of the 10 neurons that showed a decrease in activity during the gait modification (always in addition to an increase) exhibited this decrease in the stance phase following the passage of the lead limb over the obstacle.

The relative phase of activity of the population of neurons is illustrated in Fig. 7. Periods of relatively increased activity (with respect to the overall mean) are represented by the horizontal bars (see METHODS). Periods of phasic activity that were significantly greater than those observed during the control locomotion are represented by solid black lines and periods of phasic activity that were not significantly different from control are represented by dotted lines. The graph reveals the preponderance of increased discharge activity during swing. It also illustrates, however, that although a much smaller proportion of neurons showed significantly increased activity in stance (increase above control levels: solid lines), there was, nevertheless, a substantial total number of cells (37/70) that were phasically active at this time (i.e., total number phasically active in stance regardless of whether or not this represented an increase with respect to unobstructed locomotion). This can also be appreciated from the plot of the phase of peak activity in Fig. 7B by comparing the solid bars (period of maximal, increased, activity) with the open bars (total phasic activity).

Figure 7, D and E, illustrate the discharge characteristics of the previously recorded population of PTNs (Drew 1993) in the same manner. During the gait modifications, 57/91 neurons exhibited a significant increase in their discharge frequency when the lead forelimb stepped over the obstacle. As such, a
FIG. 6. Examples of the different types of rubral discharge observed during the gait modifications in the lead condition. A–D: 4 different neurons. The traces are organized as in Fig. 5.
TABLE 1. Times of occurrence of the periods of increased activity during the gait modifications

<table>
<thead>
<tr>
<th>No. of Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swing (63/70)</td>
</tr>
<tr>
<td>Phase I only</td>
</tr>
<tr>
<td>Phase II only</td>
</tr>
<tr>
<td>Phase I + Phase II</td>
</tr>
<tr>
<td>Phase I and Phase II</td>
</tr>
<tr>
<td>Stance (22/70)</td>
</tr>
<tr>
<td>Aligned with iC1B</td>
</tr>
<tr>
<td>Not aligned with iC1B</td>
</tr>
</tbody>
</table>

The table indicates the number of cells that showed increased activity during swing and stance as well as the phase of the increased activity within each of these divisions. Note that the total comes to more than 70 as many cells showed multiple periods of increased activity.

much smaller percentage of the PTNs with forelimb receptive fields showed increased activity during the gait modification (57/91, 63%) than did the red nucleus cells (70/72, 97%). In addition, using the identical analysis methods to those used for the rubral population, only 14/57 (25%) of the motor cortical cells showed multiple periods of phasic activity during the step cycle, compared with 51/70 (73%) of the red nucleus cells. Further, inspection of Fig. 7, D and E, shows that a larger proportion of cells in the motor cortex discharged before the gait modification than in the red nucleus, and many of these started to increase their discharge frequency relatively earlier than did the RN cells. Nevertheless, comparison of the time that the maximal activity occurred during the modified cycles shows that, as for the red nucleus cells, most PTNs discharged maximally in the swing phase of locomotion (Fig. 7, D and E), albeit with a noticeably increased dispersion.

Comparison of the peak discharge during the steps over the obstacle (Fig. 7C) with that obtained during control locomotion (Fig. 4C) showed that 52/70 (74%) of the cells now had peak discharges of >100 Hz compared with 28/66 (42%) during control steps. Moreover, 24/70 (34%) of the rubral neurons had discharge frequencies >200 Hz during the gait modifications compared with 10/68 (15%) in the control condition. Under the same condition only 5/57 PTNs discharged at frequencies >100 Hz during the gait modification.

Discharge activity during gait modifications: trail condition. In the trail condition, the forelimb contralateral to the recording site is the second to step over the obstacle. As we have previously illustrated (Drew 1993; Lavoie et al. 1995), in this condition, the biomechanical constraints placed on the limb are different from those in the lead condition. In particular, the placement of the paw of the trail limb with respect to the obstacle requires that the cat initiate the gait modification by producing a large retraction of the shoulder that draws the forelimb backward and away from the obstacle. This movement is caused, in part, by a substantial increase in the level of activity in shoulder retractor muscles such as the TrM (see Fig. 8D, right). There is also a strong activation of the wrist dorsiﬂexor muscles at this time. Subsequently, as the limb is brought forward over the obstacle, the EMG activation patterns are similar to those observed during unobstructed locomotion. Overall 63/69 rubral neurons that increased their discharge activity in the lead condition, also showed increased activity during at least one part of the step cycle during the trail condition (1 cell was not examined in this condition). As in the lead condition many neurons exhibited multiple increases in activity.

Most of those rubral neurons that showed a single increase in activity during phase I or phase II of the swing phase in the lead condition either showed a single increase in activity at this same phase in the trail condition, or showed no change in activity. One half (6/12) of the phase I neurons showed changes similar to those illustrated in Fig. 8A in which the single period of activity that occurred at the onset of swing in the lead condition was phase advanced in the trail condition. In all six of the cells that discharged in this manner, the peak discharge activity of the cell was greater in the trail condition than in the lead one. The remaining cells in this group showed no change in activity during the swing phase of the gait modification during the trail condition, but three of them discharged earlier in the cycle (early).

Two examples of phase II neurons are illustrated in Fig. 8B. Both of these neurons increased their discharge late in the swing phase during the lead condition but discharged prior to coCIB onset in the trail condition, at a similar time to the phase I cell illustrated in Fig. 8A. An additional 2/12 modified their discharge activity in a similar manner while another 2/12 maintained their temporal relationship with the period of activity in EDC that occurs at the end of the swing phase. The remaining 6/12 cells in this group showed no increase in discharge activity over the control levels in this trail condition.

The discharge activity of those cells that showed multiple increases of activity during swing (classified as phases I and II) showed changes in activity during the trail condition that were compatible with those described above for the separate populations of phase I and phase II cells. Seven (7/23) of these rubral neurons also showed two increases in activity in the trail condition with the initial burst occurring prior to the onset of coCIB as for the phase I cells and the second burst occurring at the end of swing as for the phase II cells. A further 15/23 showed only a single increase of activity in the trail condition; this was always prior to the onset of activity in the coCIB.

The changes in discharge activity during the trail condition of those cells that exhibited a single increase in activity throughout the swing phase (phase I + II) were more complex. The most striking characteristic of these cells was the change from a single large envelope of increased activity during lead to a pattern of smaller, multiple increases of activity in the trail condition. In the example illustrated in Fig. 8C, for example, the rubral neuron showed three clear peaks of activity just prior to and during the swing phase in the trail condition, although the level of activity was much smaller than in lead condition. The earliest peak of activity, as described for the other classes of neuron, occurred prior to the onset of activity in the coCIB. Four (4/16) of the phase I + II neurons discharged in this manner, a further 4/16 showed two clear peaks in the trail condition and 5/16 showed a single, short peak of activity, again normally prior to the onset of activity in the coCIB.

Figure 9 summarizes the changes in phase and peak amplitude that were observed for the subpopulation of neurons that were active in the swing phase during the lead condition and that also showed an increase in activity during the swing phase in the trail condition. In agreement with the data shown in the individual examples illustrated in Fig. 8, inspection of Fig. 9A confirms that, in the trail condition, the majority of swing-related cells showed a clear change in the phase of the step...
FIG. 7. Periods of activity of the neuronal discharge during the steps over the obstacle (lead condition). The data are organized in the same manner as in Fig. 4 with the following exceptions. A and D: solid black lines indicate periods of phasic activity that were significantly different from control values according to the criteria given in METHODS while dotted lines indicate periods of phasic activity that were not significantly different from control (see text). B and E: shaded bars indicate the phase of peak activity of the cell while open bars indicate the peak periods of all periods of activity, regardless of whether they were significantly different from control or not.
FIG. 8. Comparison of discharge activity in the lead and trail conditions. The data are organized as in Fig. 5. The EMG data in D are taken from the cell illustrated in C. Note that in the lead condition the left limb (coClB) precedes the right limb (iClB), whereas in the trail condition the reverse is the case.
cycle at which the initial peak of activity was observed. When plotting initial peak phase in the lead condition against initial peak phase in the trail condition, the majority of the data points were located in the bottom right quadrant of Fig. 9A. This illustrates that the initial peak was positive (subsequent to coClB activity) in the lead condition and negative (prior to coClB activity) in the trail condition. This is what we expect on the basis of the change in the pattern of EMG activity in the trail condition (Fig. 8D). As indicated in the text in the preceding paragraphs, a small number of cells maintained a positive relationship in both conditions; these are represented in the upper right quadrant.

Peak discharge frequency in the trail condition was generally decreased compared with the lead condition as can be seen from Fig. 8, B and C and Fig. 9B. In the latter plot, the majority of the cells are to be found below the diagonal line that indicates equivalence of peak discharge frequency. There are, however, a number of cells that lie above the line of equivalence and represent cells that increase their activity in the trail condition. Most of these represent cells whose discharge was temporally related to the period of activity in the coTrM. Indeed, the five cells showing the largest increase were those phase I cells whose discharge was similar to the example illustrated in Fig. 8A.

Not included in the preceding analysis are those periods of activity that occurred during the passage of the ipsilateral limb over the obstacle. Surprisingly, none of the 13 neurons that showed increased activity coincident with the passage of the ipsilateral limb over the obstacle in the lead condition showed increased activity in the trail condition, in which it was the first limb to pass over the obstacle. Indeed, as in the example illustrated in Fig. 8C, 7/13 of these cells actually exhibited a decrease in activity at this time and the other 6/13 showed no change in their activity.

**Cells with a receptive field restricted to the hindlimbs or to the face and neck.** Twenty (20/22) of the cells with a receptive field limited to the hindlimb were phasically active during control locomotion. As for those cells with a receptive field restricted to the forelimbs, most of these (18/20) showed a period of increased discharge during the swing phase of the hindlimb and many of these (15/18) also showed a second period of increased activity during the stance phase. In some cases (5/15), this latter period of activity was temporally related to the swing period of the ipsilateral hindlimb. As for the forelimb cells, a proportion (6/18) of neurons exhibited multiple periods of increased activity during swing.

During the passage of the hindlimb over the obstacle, 17/22 (77%) cells showed increased activity at some stage during the modified cycle and 16/17 had increased activity during the swing phase of this step. In all cases, this modification occurred only as the hindlimb stepped over the obstacle; there was no change in discharge related to the passage of the forelimbs over the obstacle. Figure 10A illustrates one such cell that increased its discharge frequency throughout the period of activity of the coSrt, corresponding approximately to the hindlimb swing phase. Three neurons discharged in a similar manner to that illustrated in Fig. 10A and 9/16 showed an increase in discharge restricted to phase II. Only two neurons showed an increased discharge restricted to phase I, and only a further two neurons showed an increase during both early and late swing. Five neurons also showed increased activity during the passage of the ipsilateral hindlimb.

The other major category of cells that we recorded had a receptive field restricted to the face and/or neck. During control locomotion, 7/15 were inactive, 4/15 showed a tonic discharge pattern, and 4/15 showed a weak phasic modulation. During the gait modifications, 7/15 showed an increase in activity during the modified cycle and in 5/7 of these cells, as for the example illustrated in Fig. 10B, the discharge activity began...
well in advance of the passage of the forelimb over the obstacle. All five of these cells had a receptive field that included the vibrissae. The other two neurons showed their peak activity during phase I but also started to increase their discharge frequency prior to the onset of activity in the coSrt; these two neurons also had a receptive field that included the vibrissae.

**Discussion**

This paper provides the first detailed report of the activity of rubral neurons during both unobstructed locomotion and in circumstances that required voluntary gait modifications of the fore- and hindlimbs. During unobstructed treadmill locomotion, the majority of rubral neurons were phasically modulated, with the maximum discharge frequency occurring during the swing phase of locomotion of either the contralateral forelimb or of the contralateral hindlimb. During the voluntary gait modifications, a majority of phasically modulated cells showed significant increases in their discharge frequency during the passage of either the fore- or hindlimbs over the obstacle.

**Database and methodological considerations**

Although, for technical reasons, only a few cells could be specifically identified as spinal projection neurons, the majority of the cells that we recorded had large action potentials that could be recorded over distances of several hundreds of micrometers and were, therefore, probably generated by neurons with a large soma. As most, if not all, large neurons in the feline red nucleus project to the spinal cord (Hayes and Rustioni 1981), it is likely that the majority of the cells that we recorded were rubrospinal and would, therefore, exert direct influences on spinal interneurons.

As in our motor cortical studies (Drew 1993; Widajewicz et al. 1994), we initially classified our population of cells into fore- and hindlimb related cells based on their receptive fields. That this is an appropriate method of classification for rubral neurons is suggested by our results showing that cells with receptive fields restricted to the forelimbs discharged only during the motor adjustments of the forelimbs, while those with receptive fields restricted to the hindlimbs discharged only during the motor adjustments of the hindlimbs. Moreover, our results showing that none of the cells with receptive fields restricted to the forelimbs could be activated from the lumbar spinal cord suggest that the majority of these rubral neurons exerted an influence primarily on either forelimb or hindlimb musculature. This segregation of motor function is in agreement with previous anatomical and physiological studies on the topography and input/output relationships of rubral neurons (Eccles et al. 1975a,b; Ghez 1975; Nishioka and Nakahama 1973; Pedel et al. 1972; Pompeiano and Brodal 1957; Rho et al. 1999; Robinson et al. 1987) and particularly with the finding of Shinoda et al. (1977) that only a few of the rubral neurons (2/40) that projected to the cervical cord also sent an axon to lumbar (L4) levels.

**Rubral activity during locomotion**

**Unobstructed treadmill locomotion.** Almost all (92%) rubral neurons with a receptive field restricted to the fore- or hindlimb showed a phasic modulation of their discharge activity during unobstructed treadmill locomotion with the peak discharge of the majority of the modulated neurons (89% forelimb and 90% hindlimb) centered around the swing phase (phases: 0.9–0.4). These results, in general, conform with the data of Orlovsky (1972a), who recorded the activity of rubrospinal cells projecting to the lumbar cord in thalamic cats walking on a treadmill. In those experiments, he reported that the majority of neurons (83%) were also phasically modulated during locomotion and that most (62% of the total) discharged during the swing phase of locomotion. However, in comparison to the results reported by Orlovsky (1972a), we found a larger number of our neurons (55% of modulated neurons compared with 13% in his study) discharged both during swing and stance. It is possible that this difference in the two studies might be related to differences in functional control over the hindlimb as compared with the forelimb. However, we also found a high proportion of neurons discharging during both
swing and stance in our smaller population of hindlimb related neurons. As such, it is more likely that this difference reflects either differences in the preparations used or differences in the resolution of the analytical techniques. The latter reason probably also explains the lack of any mention of cells discharging in multiple bursts during the swing phase in Orlovsky’s report.

Inspection of the discharge patterns of the rubral neurons recorded in this study compared with those previously reported for cortical neurons during unobstructed treadmill locomotion (Armstrong and Drew 1984; Drew 1993) indicates several points of similarity and difference. The most obvious similarity is that a large percentage of neurons in both structures were phasically modulated during locomotion and discharged most intensely during the swing phase of the step cycle, suggesting a basic complementarity in function. This would also be in agreement with the known anatomy and physiology of these two pathways in the cat that shows that each exerts its primary influence on the motoneurons of physiologic flexor muscles of the contralateral fore- and hindlimbs (Armstrong and Drew 1985; Ghez 1975; Hong et al.; 1969; Illert et al. 1976; Rho et al. 1999). Nevertheless, compared with the characteristics of the rubral neurons that we listed in the preceding paragraphs, the majority of the cortical neurons (81%) showed only a single peak of activity during the step cycle and few exhibited multiple peaks of activity during the swing period. As we will discuss more fully in the following sections, these basic characteristics suggest that the motor cortex exerts a more discrete control over muscular activity in different parts of the limb during locomotion than does the red nucleus.

VOLUNTARY GAIT MODIFICATIONS. General comments. Most rubral neurons increased their discharge frequency during the gait modifications required to step over the obstacles. In general, the basic characteristics of the discharge activity in individual cells, including the preponderance of phasic activity centered around the swing phase of locomotion, the presence of neurons that discharged during both swing and stance and particularly the presence of multiple peaks of activity in individual neurons during swing were similar during unobstructed locomotion and during voluntary gait modifications. This suggests that the red nucleus, like the motor cortex, contributes both to the production of the base locomotor rhythm and to the increase in the level of the EMG activity when the cats need to modify their gait. However, many neurons did not simply show a change in the magnitude of their activity but also exhibited changes in their relative phase of activation (see Figs. 5B) or showed phasic activity during the gait modification where none was present during the unobstructed locomotion (see Fig. 6). This latter characteristic resulted in neurons exhibiting more phasic peaks of activity during the gait modifications than during the unobstructed locomotion. These changes in the magnitude, duration and timing of the cell discharge patterns, together with the appearance of new periods of neuronal activity during the gait modification, suggests that the red nucleus, like the motor cortex, contributes to the specification of the appropriate spatiotemporal EMG activation patterns that are required to produce the change in limb trajectory required to step over the obstacle (see also Fig. 11).

As for the motor cortex, the major changes in neural activity in the red nucleus were observed during the swing phase of locomotion although it should be emphasized that this might be due to the fact that our task requires few modifications of EMG activity during the stance phase. We cannot rule out the possibility that both the motor cortex and the red nucleus might make a more substantial contribution to the modification of extensor muscle activity in a locomotor task that specifically challenged this aspect of the step cycle.

Activity during the swing phase. The essential characteristics of the discharge patterns observed in those rubral neurons that increased their discharge frequency during phase I or II of the swing phase of the modified cycle are summarized in Fig. 11 where they are compared with those that we have previously described for the motor cortex (Drew 1988, 1993; Drew et al. 1996) in identical conditions.

Overall, there are broad similarities in the discharge patterns of these two populations as one might expect given the complementary roles that these two structures are proposed to play in the control of voluntary movement (Kuyper 1963; Martin and Ghez 1988; Martin et al. 1993) (see also paragraphs in the following text). In both structures, for example, the period of peak activity in different populations of neurons was distributed throughout the modified swing phase. In a broad perspective, increased discharge activity was seen either during the time that the limb was being lifted above and over the obstacle (phase I) or during the preparation for landing on the other side (phase II). Among those rubral neurons that discharged during phase I, some exhibited their peak discharge activity at the beginning of the swing phase when the TrM is activated to initiate shoulder retraction to lift the limb from the support surface, whereas others discharged slightly later when elbow flexors, such as the Br, were activated to raise the paw above the obstacles or later still when wrist dorsiflexors muscles, such as the ECR, raised the paw. Neurons that increased their discharge activity during phase II showed strong temporal relationships with the period of later activity in the wrist dorsiflexor (EDC) which contributes to the stability of the paw during contact with the treadmill. The similarity in the patterns of activity in the neurons recorded from these two structures suggests that the rubrospinal system, like the corticospinal system, is implicated in specifying the appropriate sequential changes of EMG activity that are required to produce the requisite gait modification.

Nevertheless, inspection of Fig. 11 clearly shows that the signals originating in the red nucleus are not identical to those originating from the motor cortex. There are differences in the relative proportion of neurons active at different times during the gait modification, in the rate of discharge, in the proportion of modified neurons active at different times during the swing phase, and in the patterns of activity observed in neurons in the two structures. Among these differences, we consider that the most important, from a functional point of view, is the existence of the large proportion of rubral neurons that show multiple periods of increased activity during the gait modification. In our population of rubral neurons, 37% exhibited two periods of increased activity during the swing phase of the modified cycle. In all of these neurons, the second period of increased activity occurred during phase II, just prior to paw contact, while the initial period of activity occurred at varying times during phase I. In contrast, using the same analytical methods and criteria, none of the neurons recorded in the motor cortex showed such multiple periods of increased activity during swing.
Such a double (and occasionally triple) period of activation may imply that any one neuron modulates the activity of a given group of synergistic muscles at two different periods of the step cycle. For example, several muscles, including the wrist dorsiflexors ECR and EDC (see e.g., Figs. 3B, 5, and 11), discharge in multiple bursts when the cats step over the obstacles and the rubral cells might well contribute to the regulation of each of these periods of activity. However, it should be noted that the first period of activity in these cells was very variable (see error bars in Fig. 11) and, in different cells, covaried with the activity of the shoulder retractors, the elbow flexors, or the wrist dorsiflexors. We therefore cannot rule out an alternative explanation that some of these neurons with multiple discharge may also contribute to the regulation of different muscle groups, including both proximal and distal muscles, at different times during the step. Although our methodology does not allow us to distinguish between these two possibilities, the results clearly indicate that a substantial proportion of rubral neurons contribute to the regulation of muscle activity both during the transport phase of the step, as the limb is brought above and over the obstacle, as well as during the subsequent placement phase, as the limb is prepared for contact with the support surface.

The descending signal carried by rubral neurons might therefore provide two different types of message. One, carried by neurons discharging in multiple bursts, would be a signal that could be used as an aid to coordinating the activity of different periods of activation in a given group of muscles, or different groups of muscles active at different times, during the swing phase. The other, carried by those rubral neurons discharging with a single burst of activity, as well as by the majority of cortical neurons, would allow more independent modification of small groups of muscles at specific periods of the swing phase allowing the trajectory to be finely adjusted to the requirements of the task. In this respect, another of the differences in the characteristics of the rubral and cortical populations may also be critical. Namely, the finding that all of the rubral neurons that we recorded showed increased activity during the gait modifications. In contrast, only 63% of the cortical population showed increased activity, whereas 30% showed a decrease in activity levels. Similar findings have also been noted by others (Amalric et al. 1983; Otero 1976). These characteristics would also be compatible with the idea that the motor cortex exerts a fine balance over the modifications in EMG activity that are required both by increasing and decreasing the level of activity of the spinal interneuronal pathways involved in regulating the structure of the step cycle.

**During swing and stance.** In addition to those cells that discharged in multiple bursts of activity during swing, there was also an appreciable proportion (53%) of cells that discharged during both swing and stance during the steps over the obstacle. This was something that was less frequently observed in the population of motor cortical cells in which only 16% discharged during both phases during the gait modification. These differences also suggest differences in the functional role of the two structures in the control of locomotion. It is possible that this dual activity is another indication that the red nucleus is involved in signaling more general or coordinative changes in EMG activity in the limb; in this case, not only between different groups of flexor muscles active during the swing phase but also of the extensor muscles active during stance. Such a widespread influence over flexor and extensor muscle activity is compatible with the available physiological evidence showing that rubrospinal volley may influence both flexor and extensor motoneurons (Belhaj-Saif et al. 1998; Cheney et al. 1991; Ghez 1975; Hongo et al. 1969, 1972; Pinter et al. 1982; Powers et al. 1993; Rho et al. 1999). Moreover, microstimulation at different loci within the red nucleus during locomotion frequently evoked transient increases in extensor activity during the stance phase of locomotion and transient increases of flexor muscle activity during the swing phase (Rho et al. 1999).

**During swing of the ipsilateral forelimb.** A substantial number of the rubral neurons that we recorded discharged in a relatively discrete pattern during the stance phase, both during unobstructed locomotion (e.g., Fig. 3) and following the step over the obstacle (e.g., Fig. 6). Although these rubral neurons might be considered to be involved in regulating extensor muscle activity during this period, most of the major extensor muscles that have been recorded are active throughout the stance period [see e.g., TriL in Figs. 2 and 5 and Drew and Rossignol (1987) for other extensor muscles]. Moreover, these discrete bursts of neuronal activity were invariably active at the same time as iClB, leading us to suggest a role for some rubral neurons in contributing to the swing phase of the ipsilateral limb during locomotion in cats.

The fact that so many rubral neurons showed a period of activity coincident with the period of ipsilateral swing was initially unexpected given the fact that the rubrospinal pathway is completely crossed (Massion 1967). However, post hoc, the result is not incompatible with our current knowledge of the anatomy and physiology of this system. For example, despite the entirely crossed nature of the rubrospinal tract, Eccles et al. (1975a,b) demonstrated that rubrospinal neurons received in-
put from the ipsilateral limb that was almost as strong as that from the ipsilateral one. Others (Batson and Amassian 1986; Eccles et al. 1975a,b; Ghez 1975; Nishioka and Nakahama 1973; Padel et al. 1988; Vinay and Padel 1990) have also described bilateral afferent input to the RN, although most of these have also emphasized that the receptive fields on the contralateral limb are more sensitive than those on the ipsilateral limb.

With respect to the output of the RN, we have previously shown that microstimulation of this structure in these same animals produced weak activation of ipsilateral forelimb flexors, possibly via commissural interneurons (although we cannot discount the possibility that we may also have activated decussating fibers). It is therefore quite possible that some of these rubral neurons modulate activity in both the contralateral and ipsilateral forelimbs. Taken together with the earlier discussion concerning the multiple bursts during contralateral swing, and during swing and stance, these results are compatible with the viewpoint of a system involved in coordinating activity during locomotion, not only of intralimb activity, but also, to a lesser extent, interlimb.

However, if this burst of activity is indeed related to activity of the ipsilateral limb, as we suggest, then it is somewhat surprising that so few of these neurons showed an increase in activity in the trail condition. Indeed, in many cases, the rubral neurons even exhibited a decrease in activity in this condition despite the large increase in activity in iClB (see Fig. 8). The reasons for this are unclear but might imply an organization whereby the RN regulation of the ipsilateral limb is subservient to the activity in the contralateral limb. That is, activity in the lead limb would be regulated only by the contralateral RN (and MC), whereas the activity in the trail limb would be influenced both by the contralateral RN and, to a lesser extent, from the ipsilateral side. This again would speak for some role in interlimb coordination.

Integration with the locomotor rhythm

Although the multiple periods of activity observed in some of these rubral neurons may provide a neural mechanism for ensuring that periods of increased EMG activation at different joints during different phases of the movement are produced at the appropriate time, it does raise the problem as to how the nervous system ensures that each period of increased activity acts only on the appropriate muscles. For example, why does the period of increased activity during stance not produce inappropriate activation of muscles that are activated only during swing and vice versa? There are several possible explanations.

First, during locomotion, this pattern of activity is being superimposed onto the basic locomotor rhythm that itself is capable of determining much of the underlying structure of the step cycle. For example, during swing, microstimulation preferentially activates physiological flexor muscles, whereas during stance, it modulates the activity in physiological extensors (Rho et al. 1999). The rhythmical changes in polarization in these different groups of muscles therefore provides a simple method to allow single neurons to regulate activity in different groups of muscles. A similar mechanism may also function at finer resolution and provide a means for single neurons to differentially regulate the activity of different muscles during the swing phase. For example, our microstimulation study (Rho et al. 1999, Fig. 5) shows that stimulation at a single site in the red nucleus evoked maximal responses in the TrM, Br, and EDC at different times during the swing phase. Maximal responses in the TrM were evoked at the onset of the swing phase while maximal responses in the EDC were evoked at the end of the swing phase. It is therefore possible that the phasic modulations of the level of excitability of spinal interneurons is sufficient to induce some functional organization of the descending signals originating in the red nucleus. We have suggested (Drew 1991; Drew et al. 1996; Prentice and Drew 1997) that such temporal sculpting may also provide a means of focusing the effects of the cortical descending command during locomotion, thus partially compensating for the large spatial distribution of the terminal arbors (Futami et al. 1979). Similar spinal mechanisms would also contribute to the appropriate integration of the signals modifying activity in both contralateral and ipsilateral limbs.

In addition to these passive mechanisms, the final expression of the descending signal will also depend on the integrated activity of the overall population of neurons providing input to the interneuronal circuits controlling the limb, including the large population of cortical and rubral neurons that increase their discharge in a single, discrete part of the modified cycle. This concentrated signal, at specific phases, will likely have the effect of facilitating transmission in spinal circuits to certain muscle groups while decreasing transmission in others. In this respect, the modification of muscle activity produced by the rubral neurons discharging in multiple bursts may be contingent on the activity of those neurons with more discrete activity. This is similar to the suggestion that we have previously made (Prentice and Drew 2001) that the efficacy of descending reticulospinal volleys may be contingent on the activity in other descending pathways, including the corticospinal tract.

Source of modulation

The major afferent input to the red nucleus is from the interpositus nucleus (Massion 1967; Toyama et al. 1968) with weaker inputs from the motor cortex (Padel et al. 1973; Tsukahara et al. 1967, 1968) and from the spinorubral pathway (Vinay and Padel 1990). All of these may serve to modulate the activity of the rubral neurons although the greater strength and faster time course of the excitatory postsynaptic potentials (EPSPs) produced by stimulation of the interpositus nucleus with respect to those observed following stimulation of the motor cortex (Tsukahara et al. 1967) suggests that the cerebellar input is likely to be the major determinant of the pattern of activity observed in the rubral cells. In this respect, it is important to note that recordings of interpositus neuronal activity in both the decerebrate (Orlovsky 1972c) and in the intact cat (Armstrong and Edgley 1984) show clear phasic activity in these cells, particularly during the swing phase of unobstructed locomotion and that cooling of the interpositus nucleus during locomotion leads to a reduction in the amplitude of the flexion phase of locomotion (Udo et al. 1979, 1980), presumably due to disinhibition of the interpositorubral pathway. In a complementary manner, injection of muscimol into the interpositus nucleus also leads to hypoflexion and to some changes in EMG timing patterns during the swing phase of locomotion (unpub-
lished observations) (see also Bracha et al. 1999; Rathelot et al. 1996).

It seems probable that the interpositus nucleus is also the primary source of input to the red nucleus during the voluntary gait modifications, although in this case, there is no direct evidence to support this supposition. Indeed, the only study that has examined interpositus discharge during a voluntary locomotor task reported little change in activity compared with unobstructed treadmill locomotion (Armstrong and Marple-Horvat 1996). However, as the authors emphasized, only a small number of neurons were examined, and direct comparison of the discharge activity during ladder and treadmill locomotion was not possible. Moreover, experiments in which injections of muscimol have been made into the interpositus nucleus showed a clear disruption of the ability of cats to successfully step over obstacles (Rathelot et al. 1996), or to walk from rung to rung of a horizontally positioned ladder (Bracha et al. 1999), suggesting a contribution from the interpositus nucleus in modulating this activity. Last, there is abundant evidence from experiments in cats and primates trained to make forelimb reaching movements to suggest that the interpositus nucleus is critically involved in the execution of such voluntary movements (Burton and Onoda 1978; Fortier et al. 1989; Gibson et al. 1998; Harvey et al. 1979; Martin et al. 2000; Milak et al. 1995; Thach 1970; van Kan et al. 1994).

The role of the motor cortex in determining the level of activity in rubral cells is less clear. Although we have already discussed the strong resemblance in the pattern of activity observed in these two structures during the gait modification, most of the synaptic contacts between cortical cells and rubral neurons are found on the distal dendrites and produce only small and slow EPSPs (Tsukahara et al. 1967). It seems more likely that cortical input would modulate rubral activity rather than being a prime determinant of the pattern of discharge. This would be in agreement with the fact that the pattern of activity of the rubral neurons, although not the level, is quite similar in the intact and the decerebrate cat during unobstructed treadmill locomotion. Nevertheless, we cannot rule out the possibility that the multiple periods of activity during the swing phase might be the result of the combined influence of the cortical and cerebellar inputs.

The role of the direct spinorubral pathway in determining the modulatory activity of rubral neurons is also unclear. Although peripheral afferent input is transmitted directly, and at short latency, to rubrospinal neurons by this path, Orlovsky (1972a) demonstrated that the modulatory pattern of rubral neurons is mostly lost following cerebellectomy (see, however, Vinay et al. 1993), suggesting that the direct spinorubral pathway is not a major determinant of the pattern of rubral activity during locomotion.

Comparative aspects

The fundamental results obtained during this locomotor study are very similar to those obtained by other research groups examining the role of the red nucleus in the control of reaching movements (see introduction for references), suggesting a generally similar mode of control of reaching and voluntary gait modifications. At a general level, the discharge rates observed during the gait modification were generally as high as those observed during reaching movements in both cats and primates, suggesting a similar level of influence over the muscle activity in the two different types of activity. Moreover, in agreement with the results obtained in primates (Gibson et al. 1985a,b; Mewes and Cheney 1991; Miller et al. 1993; Sinkjaer et al. 1995; van Kan and McCurdy 2001) the temporal aspects of the discharge activity in a majority of rubral neurons (56%) was best related to the activity of physiological flexor muscles of the wrist and digits, such as the ECR and EDC. This is also in agreement with a recent study in rats (Jarrett and Hyland 1999) showing that most rubral neurons discharged toward the end of a reaching task corresponding to extension of the wrist. This suggests a parallel in the importance of the red nucleus in controlling the wrist during coordinated reaching and grasping movements and during locomotion. It is probable that a locomotor task in which paw placement needed to be more precisely controlled might show an even greater modification of the neuronal discharge at this phase of the step cycle. However, while these results suggest a powerful contribution to the control of distal musculature, it should nevertheless be emphasized that a similar proportion of neurons might influence proximal musculature, some of them simultaneously. As emphasized by several authors (Belhaj-Saif et al. 1998; Cheney et al. 1991; Gibson et al. 1998; Mewes and Cheney 1991), the rubral projection likely contributes to the control of both proximal and distal musculature and may fire preferentially during coordinated movements of the whole limb that are associated with control of the hand (Gibson et al. 1998; Mewes and Cheney 1994; Miller et al. 1993; van Kan and McCurdy 2001).

One major difference between the results of this study and those of most other studies examining reaching movements is the apparent absence of neurons showing multiple periods of increased activity during reaching. In most studies on reaching, neurons are described as discharging in a single burst of activity during the transport period of the reach, although a recent paper on reaching in the rat (Jarrett and Hyland 1999) shows multiple periods of activity similar to those described in the present paper. Similarly, we are aware of no reports of rubral neurons discharging during ipsilateral limb movements in primates. Whether these are true differences between rubral activity in cats and rats compared with primates or simply due to differences in task or to different analytical techniques is not clear.

Concluding remarks

During locomotion, the rubrospinal system is normally considered to play the relatively simple role of adjusting the overall level of flexor muscle activity (Orlovsky 1972b). As stated in the introduction, this view is based primarily on the lack of any major deficits following lesion of the nucleus, partly on the fact that neuronal activity in this structure during locomotion had been previously recorded only during locomotion in the decerebrate animal and partly on the finding that stimulation of this structure has no effect on cycle timing. In contrast, the results presented in this study suggest that the red nucleus plays an important role in the regulation of the locomotor cycle, especially during conditions that require adjustment of the spatiotemporal muscle activity patterns in the limb. In this respect, as detailed in the preceding text, the results agree with the view obtained from neuronal recording studies.
in animals trained to make reaching movements that the rubrospinal system, together with the corticospinal pathway, provides a major part of the descending signal that is used to modify voluntary movement. Nevertheless, as discussed in the preceding paragraphs, the functions of the two systems are not identical, and, as argued by others (e.g., Martin and Ghez 1988), they likely play complementary roles in the control of movement determined by their afferent inputs and their spinal projection patterns.

In particular, the multiple periods of increased activity during the step over the obstacle suggest that individual rubral neurons, in contrast to motor cortical neurons, contribute to the regulation of muscle activity in both phase I (the transport phase) and phase II (the placement phase). This suggests a contribution to the regulation of intralimb coordination. In addition, the multiple activation of some neurons during swing and stance and time-locked to contralateral and ipsilateral swing suggest that some neurons may also play a role in interlimb coordination at least during locomotion. As such, the general characteristics of the rubrospinal tract fall between the high degree of specificity shown by the corticospinal tract and the more diffuse action of the reticulospinal tract. We suggest that these general characteristics may be related, in part, to the evolutionary lineage of the red nucleus. The red nucleus evolved with the first land animals and has been suggested to be tightly linked to the control of the limbs during locomotion (Keifer and Houk 1994; ten Donkelaar 1988). In these more primitive animals, limb movements were relatively stereotyped, and there was probably little requirement for a descending control system that would allow specific and fractionated control of individual joints. On these general characteristics there would then be superimposed further evolutionary changes related to the different behavioral requirements of different species and the level of development of the corticospinal tract. In the cat, these evolutionary pressures have resulted in two complementary systems involved in the voluntary control of limb movement with both pathways providing a descending signal that can be used to regulate activity in small groups of synergistic muscles, particularly in the distal limb, but with the rubrospinal system providing an additional signal that might aid in the coordination of intra- and interlimb coordination.

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REFERENCES


