their antipsychotic effects, may be contraindicated during recovery from brain injury because they block catecholamine receptors (*16*) and may slow the recovery of function.

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6. Beam-walking was studied since rats with motor cortex lesions show little movement dysfunction on a broad, flat surface but display a marked deficit when placed on a narrow beam. In the first days after unilateral ablation of the motor cortex, the contralateral limbs (especially the hind limb) hang off the beam and the animal may fall. In training and testing, the animal was placed on one end of a wooden beam (122 by 2.5 cm), close to the sources of noise and light. The noise was terminated after the animal traversed the beam into a dark goal box (24.5 by 18 by 20 cm). Training consisted of two trials every other day until the animal could run the length of the beam with no more than two foot slips, a criterion reached within 10 days.
7. After the experiments the subjects were anesthetized with pentobarbital and perfused with saline and Formalin. The brains were removed and photographed, and the lesions were verified by studying frozen sections stained with thionine. Occasionally a lesion involved the dorsal striatum and hippocampus. In most cases the entire motor cortex was removed from one hemisphere [R. D. Hall and E. P. Lindholm, *Brain Res.* **66**, 23 (1974)]. There were no systematic differences among the groups in the amount or area of brain removed.
8. The experimenters had extensive practice using the rating scale before the experiments began. There was a 98 percent agreement in ratings between the informed experimenter and the uninformed experimenter, and the few disagreements never involved more than one category. On the rating scale, an animal received a 7 if it traversed the beam normally with no more than two foot slips; a 6 if it traversed the beam and used the affected limbs to aid more than 50 percent of its steps along the beam; a 5 if it used the affected limbs in less than half of its steps along the beam; a 4 if it traversed the beam and at least once placed the affected hind paw on the horizontal surface of the beam; a 3 if it traversed the beam while dragging the affected hind limb; a 2 if it was unable to traverse the beam but placed the affected hind limb on the horizontal surface of the beam and maintained balance; and a 1 if it was unable to traverse the beam and could not place the affected hind limb on the horizontal surface.
9. A repeated measures analysis of variance (ANOVA) was performed to compare the groups. For the animals given practice, amphetamine had a significant effect on recovery [$F(1, 27) = 20.683$, $P < .001$] (Fig. 1A), as did haloperidol [$F(2, 25) = 6.034$, $P < .007$] (Fig. 1C). These drugs did not affect recovery in the restrained animals (Fig. 1, B and D). The same analysis performed on the dose-response data gave $F(4, 45) = 4.16$, $P < .01$. Newman-Keuls tests were conducted to make specific comparisons between groups at different times after drug administration; these probability values are reported in the text.
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Velocity Signals Related to Hand Movements Recorded from Red Nucleus Neurons in Monkeys

Abstract. *Neural activity of the red nucleus was studied in monkeys trained to operate devices requiring shoulder, elbow, wrist, hand, or finger movements. Single cell activity was more closely related to movements of the hand and fingers than to movements of the other joints. Discharge consistently preceded movements by a constant time interval; duration of discharge was highly correlated with the duration of movement; and discharge rate was highly correlated with movement velocity. These data suggest a role for the rubrospinal pathway in the initiation and control of hand movements.*

The magnocellular division of the red nucleus (designated RNm) is the origin of one of several large-fiber tracts that descend from the brainstem to the spinal cord. Signals from the cerebellum and motor cortex converge on RNm neurons to produce motor commands. These commands are then conducted in the rubrospinal tract to propriospinal neurons, segmental interneurons, and, in primates, directly to motor neurons (*1*). The specific nature of the motor commands transmitted by the RNm is poorly understood. We investigated the particular categories of movement controlled by the RNm and also the temporal and parametric features of these nerve signals.

Since lesions of the rubrospinal pathway result in deficits in the control of distal joints of the extremities in both monkeys and cats (*2*), we were interested in knowing how the discharge of RNm neurons varies as the animal performs movements of the hand and fingers. Microelectrode recording has been used in studies in which trained monkeys (*3–5*) and cats (*6*) performed tasks that emphasized motion about more proximal joints, from the wrist to the shoulder, and limited to a single category of movement. We have now examined the relations of the RNm neurons to hand and finger movements using several manipulanda in order to compare relations at different joints (*7*).

A device, which we call the twister (Fig. 1A), operates like a motorcycle throttle. Twister rotation is sensed by a potentiometer circuit that drives a position trace on a visual tracking display. A pair of horizontal traces on the tracking display designate a target zone. A monkey is required to manipulate the position trace between the horizontal traces by rotating the twister, in order to obtain a liquid reward. The monkey's head and body are restrained, and a tungsten microelectrode used for recording (0.2 to 1 megohm impedance) is positioned in the animal's brain with a standard chamber and microdrive (*8*). Operation of the twister requires a coordinated hand movement, mainly involving the action of finger and wrist muscles (confirmed by intramuscular electromyographic recording). Other movements were tested with the same tracking display and different manipulanda. One device required push-pull movements of the whole limb, and other devices were designed to isolate movements of the fingers, thumb, wrist, elbow, or shoulder. During the study of each neuron, we supplemented quantitative observations on one to four devices with a qualitative examination of the neural activity associated with reaching for and manipulating food objects.

Our results are based on 327 single-unit recordings from well-characterized RNm neurons in two male rhesus and one male cynomolgus monkey. During

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the daily recording sessions, which were spaced over several months, we relied on stereotaxic coordinates, the characteristic eye movement–related discharge of the oculomotor nucleus, and other physiological landmarks to find RNm neurons. Our recording sites were later confirmed histologically by the location of electrode tracks and small electrolytic lesions made by passing current through the recording electrode at selected sites (Fig. 1B). The RNm units had large action potentials (0.4 to 2 mV) and spontaneous rates in the range 0 to 50 pulses per second; the neurons discharged vigorously at rates that often exceeded 100 pulses per second when the animal made appropriate movements.

Within the RNm, there was a clear separation between three somatotopic zones. Most of our recordings (230 units) were from a dorsomedial forelimb zone. On some penetrations, we encountered activity related to mouth and facial movements just dorsal to the forelimb zone (28 units). There is also a large ventrolateral hind limb zone that we sampled only partially (69 units), since we often withdrew the electrode when it entered the lower limb zone in order to minimize tissue damage. This overall somatotopy agrees well with previous anatomic, stimulation, and recording studies (*9*).

Qualitative tests with free limb movements suggested that RNm discharge is more closely related to hand and finger movements in the fore limb (77 percent of 230 units) and to foot and toe movements (*10*) in the hind limb (83 percent of 69 units) than to movement about more proximal joints. Frequently, high discharge rates were associated with even slight movements of the digits.

Using computer records obtained during tracking movements, we analyzed 137 well-isolated single units located within the dorsomedial upper limb zone. We considered a unit to be well related to a particular movement if its discharge rate was modulated by 50 pulses per second or more. With this criterion, only 5 of 22 tested units were well related to push-pull movements of the whole arm; for most of these 22 units, discharge was correlated more closely with adjustments of the monkey's grip on the manipulandum handle than with the movement about proximal joints. In tests with other manipulanda, 1 of 6 units was well related to shoulder movement, 3 of 13 were well related to elbow movement, and 47 of 117 were well related to performance on the twister.

The most consistent discharge modulations were seen during operation of the twister, which required a complex movement that involved both wrist and metacarpophalangeal joints. A crank device that required wrist movements without finger involvement resulted in no background activity in RNm; of 16 cells isolated and tested on the device, none modulated above the criterion of 50 pulses per second. Of 17 cells that gave more modulation than 50 pulses per second on the twister, 9 also gave more modulation than this criterion on tasks that isolated finger movements. Thus, finger movements are relevant for the discharge, but coordinated action of the wrist and fingers may also be important.

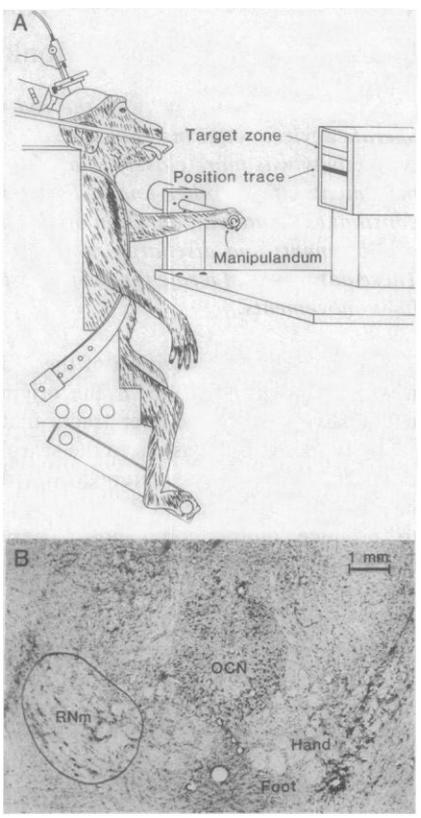

Fig. 1. (A) Monkeys were trained to track a target on a visual display using one of several manipulanda as a tracking device. The manipulandum shown here, called the twister, was our best device for provoking modulations in discharge rate of red nucleus neurons. A microdrive attached to a chamber that was mounted to the skull was used to position a tungsten microelectrode for single-unit recording. (B) A cresyl-stained frontal section taken through the midbrain at the level of the magnocellular division of the red nucleus (*RNm*). The oculomotor nucleus (*OCN*) is in the center, and the large cells ventral and lateral to the oculomotor nucleus are the left and right RNm. The light circle surrounded by a darker staining area is a lesion site where recordings were made from a cell related to foot movements. About 1 mm dorsal to this area is a lesion (less obvious in this section) placed in an area related to hand movements. The histology from all subjects showed recording sites to be in the RNm.

Unit activity displayed considerable specificity for a given task when the modulation criterion was applied. Of the 66 units tested on multiple manipulanda, 43 were tested on two devices, 20 on three devices, and 3 on four devices. Among these, 24 units were well related to performance on a single device, 13 were well related to performance on two devices, and one was well related to performance on three devices. Of the 14 instances in which unit activity was well related to performance on more than one device, 11 occurred with alternative devices for eliciting hand and finger movements. Twenty-eight units were not well related to performance on any of the manipulanda tested, even though 20 of these discharged vigorously when the animal grasped raisins or used its hand in other ways; these units thus appeared to be related to specific categories of hand movement not tested by the available manipulanda.

After a device was determined to be effective in eliciting unit activity, the temporal and parametric characteristics of the unit activity were studied. Figure 2 shows the discharge patterns of a neuron during twister movement. The movements were provoked by changes in target position that began at time zero. The target was changed stepwise in Fig. 2A, whereas it was moved at a slow constant velocity (ramp) in Fig. 2, B and C. The monkey was not particularly proficient at ramp tracking, but even trials with irregular performance (Fig. 2B) proved valuable for subsequent analysis. The traces labeled "CUSUM" (*11*) in Fig. 2 were constructed by summing the cumulative number of spikes as a function of time, minus a constant rate chosen to counterbalance the spontaneous discharge of the cell in the prestimulus interval, thereby enhancing the display of changes in spike activity. CUSUM's transform bursts of irregular activity into smooth steplike transitions and irregularly maintained tonic responses into ramplike transitions (Fig. 2A).

The onsets of bursts in discharge were readily determined from inflections in the CUSUM's (solid vertical markers in Fig. 2). These events could then be compared with the onset times of the relevant movements (dotted vertical markers). The onset of a burst preceded the onset of movement, and the lead times of 120 to 140 msec shown in Fig. 2 are typical. For a population of 32 units, the mean lead time was 124.2 ± 27.2 (standard deviation) msec. Electromyographic recordings showed that prime movers were activated approximately 55 msec before our estimate of movement onset.

Thus, the average RNm unit fired 70 msec before muscle activation, a time interval that is consistent with a role for RNm in movement initiation.

The durations of the bursts of neuronal discharge corresponded closely with movement durations. Figure 2A shows this in simplest form and Fig. 2B illustrates that the rule may apply only to the "on" direction of movement, since most well-related units displayed unidirectional firing patterns. The correspondence between burst and movement duration is illustrated by the long periods of discharge corresponding to the long periods of movement in Fig. 2C. The fact that the burst began before the onset of movement and terminated before the completion of the movement suggests that sensory feedback may not be a major causative factor in controlling RNm activity.

Discharge rate was highly correlated with the velocity of movement for many neurons [also compare (*4*, *6*)]. For example, a rate of 180 pulses per second precedes the rapid movement in Fig. 2A in comparison with the 60 pulses per second prevalent throughout the slow movement extending from 0.9 to 1.7 seconds in Fig. 2C. The intermediate velocity movements at the beginning and end of this time period were preceded by bursts of 110 and 120 pulses per second. Similar correlations between discharge rate and velocity were found for units related to finger movements and also for the few units related to elbow and shoulder movement. Of 27 well-related units that were studied over a range of velocities, the trial-by-trial correlation between discharge rate and velocity reached statistical significance ($P < .05$) for 25; the correlation coefficients for 13 of these exceeded 0.8 and the highest was 0.97. These correlations appear to be as high as those reported for premotor "burster" neurons in the oculomotor system (*12*). Analogous trial-by-trial correlations have rarely been described for the skeletomotor system, and for the cases reported (*13*), the correlation coefficients have been lower.

Comparison of CUSUM's with position traces generally demonstrated a remarkable similarity (Fig. 2, A and C), except that the CUSUM failed to reflect movements in the "off" direction (as in Fig. 2B) because of the predominant unidirectional nature of RNm burst behavior. The correlation in the "on" direction provides an additional demonstration of the close correspondence (after correction for the lead time) between RNm discharge rate and movement velocity. Since the computation of CUSUM's requires mathematical integration of the spike train, the processed signals should resemble position traces —as in fact they do.

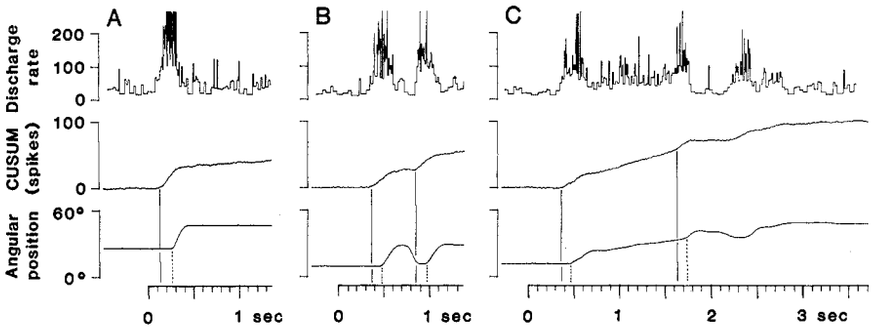

Fig. 2. Well-related single units such as this one showed burst changes in discharge rate, measured in pulses per second (upper traces), with onsets (solid vertical markers) that preceded movement onsets (dotted vertical markers) and rates that correlated with movement velocities. The middle traces in the three trials are integrated spike records, called CUSUM's; except for a directional asymmetry, these spike records closely resemble the records in the lower traces, which show the angular position of the twister. The three individual trials are in response to (A) a step target and (B) and (C) ramp targets, all starting at time zero.

The preferential relation between RNm activity and hand movements may help to explain differences between our results and the temporal correlations reported by other investigators of the primate red nucleus. The task used by Otero (*5*) required that the arm be lifted to position the hand before a button could be pushed to deliver the reward. Electromyographic recordings indicated that proximal muscles were active before distal ones; motor cortical unit activity preceded proximal muscle activity, which was then followed by red nucleus activity. Otero concluded that movements are initiated by motor cortex, but not by the red nucleus. However, the difference in timing may be the result of cortex-related proximal muscle activity preceding the red nucleus–related distal muscle activity in the task. More recent reports (*4*) indicated that RNm activity typically starts after the onset of a pronation-supination movement and tends to peak near movement termination, suggesting a role for RNm in the braking phase of movement, but not in movement initiation. One possible alternative interpretation is that this delayed timing may correlate with some associated movement, such as an adjustment of the grip of the fingers on the manipulandum handle. Cheney (*3*) reported that half of a small sample of modulated RNm units discharged before wrist movement, whereas the other half followed the onset of movement. Our conclusion that discharge consistently precedes movement agrees with some, although not all, of the findings reported for the awake cat (*6*).

In summary, our results provide three lines of support for the hypothesis that the RNm participates (presumably along with the motor cortex) in the initiation and control of hand and foot movements: (i) many units show large modulations in discharge in association with hand and foot movements and not with movements about more proximal joints; (ii) the activity of well-related units consistently precedes movement onset; and (iii) discharge rate correlates well with movement parameters, namely, the duration and velocity of movement. Since the RNm contains relatively few cells and appears to participate in a small number of specific movements, the rubrospinal pathway may be useful as a simplified model for studying the genesis and properties of descending motor commands.

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Brain Injury Causes a Time-Dependent Increase in Neuronotrophic Activity at the Lesion Site

Abstract. *A cavity was made in the brain (entorhinal cortex) of developing or adult rats, and a small piece of Gelfoam was emplaced to collect fluid secreted into the wound. The neuronotrophic activity of the fluid was assayed with sympathetic and parasympathetic neurons in culture. The results show that wounds in the brain of developing or adult rats stimulate the accumulation of neuronotrophic factors and that the activity of these factors increases over the first few days after infliction of the damage.*

The potential of central nervous system (CNS) tissue to recover from injury depends on the substances and processes that support neuron survival (neuronotrophic factors), promote the sprouting of neurites, and guide the neurites to their targets. The existence of substances with neuronotrophic, neurite-promoting, and guiding activities has been known for about 30 years. The best characterized of these molecules is nerve growth factor, which exhibits all three types of activity (*1*). The search for factors with trophic effects on central as well as peripheral cholinergic neurons has led to the description of a variety of such factors present in tissue extracts or secreted by cells in culture (*2*).

It has been suggested that, following injury to the nervous system, neurite-promoting and neuronotrophic factors are made available to facilitate repair (*3*). Evidence supporting the existence of such factors has been either rare, as in the case of the peripheral nervous system (*4*), or indirect, as in the case of the CNS (*5*).

Recently, however, a new system for nerve regeneration was used to demonstrate that neuronotrophic factors appear when the adult rat peripheral nervous system is damaged. The fluid that accumulated between the two stumps of a resected sciatic nerve and bathed the regenerating nerve structure contained trophic factors for sensory, motor, and sympathetic neurons, the contributors to the sciatic nerve (*6*). Lewis and Cotman (*7*) showed that the survival and growth of embryonic striatal tissue implanted in a cavity made in the entorhinal or occipital cortices of 3-day-old rats was much increased if the implant was emplaced 3 to 6 days after infliction of the wound. These observations suggest that factors accumulate in brain wounds which aid neuron survival and growth.

We tested this possibility by collecting the fluid secreted into brain wound cavities and measuring its ability to support neuron survival in culture. A cavity of 2 to 4 mm^3 was made in the entorhinal or occipital cortex of Sprague-Dawley rat pups (3 days old) or young adults (45 to 60 days old), and the cavity was filled with Gelfoam (Upjohn) moistened in isotonic saline solution. At various times after the operation the Gelfoam was removed and extracted with cell culture medium. The extract was tested for its ability to support the survival of dissociated neurons from ciliary (cholinergic) ganglia or sympathetic (noradrenergic) ganglia from chick embryos in the 8th or 12th day of development, respectively. The extracts were serially diluted and placed in the wells of microtiter plates. Neurons were added and the cultures were incubated for 24 hours, at which time they were fixed in 2 percent glutaraldehyde. The neurons were then counted under a phase microscope (*8*). The assay is set up so that in the absence of exogenous trophic factors, neuron survival is less than 15 percent of the maximum values obtained in the presence of nerve growth factor (sympathetic ganglia) or eye-derived neuronotrophic factor (ciliary ganglia) (*9*).

All implant surgery was performed on day 0. Extracts from the excised tissue had little or no neuronotrophic activity (Fig. 1). In the developing animals low neuronotrophic activity appeared on day 1 and increased to very high levels by days 3 and 6. In the young adults neuronotrophic activity was also present in the fluid by day 6 (little or no activity was evident earlier). The Gelfoam itself and the rat serum had no trophic activity. The extract was usually more active on sympathetic than ciliary ganglia neurons.

Trophic activity of the brain fluid was not lost by centrifugation at 250,000*g* or by dialysis but was destroyed by heating (90°C for 10 minutes) and by digestion with trypsin (incubation with 100 U for

Table 1. Effect of various treatments on the trophic activity of the Gelfoam extracts. Three to 6 days after infliction of the lesions, extracts of Gelfoam fragments from neonatal animals were pooled and 10-μl portions were titrated on the various test neurons after being subjected to a variety of treatments. Control samples were kept at 4°C for 60 minutes and then were diluted to 200 μl with modified Eagle's medium (*8*) before serial dilution and assay in the microtiter plates. For heat treatment, the portions were diluted to 200 μl, incubated for 10 minutes at 90°C, and returned to 4°C until assay time. Other portions were mixed with 50 μl of modified Eagle's medium, dialyzed against the medium for 2 hours at 4°C, and brought to 200 μl for assay. For trypsin treatment, samples were mixed with 10 μl of medium and 10 μl of trypsin stock (9700 U/mg; 1 mg/ml in medium), incubated for 60 minutes at 37°C, and mixed with 10 μl of Trasylol stock (100 U/ml) and 160 μl of medium. The final 200-μl mixture was kept at 4°C until being assayed. The same protocol was used for sample portions receiving trypsin and Trasylol at the same time. Antiserum to nerve growth factor (NGF) (1 μl blocks the activity of 5 trophic units of the factor) was added to other portions (20 μl of antiserum to 10 μl of CNS neural fluid), and the mixture was brought to 200 μl and kept at 4°C until being assayed. In this case, however, the subsequent serial dilution was carried out in the constant presence of antiserum to NGF (100 μl/ml). Trasylol or serum from unimmunized rabbits had no effect on trophic activity.

Treatment	Trophic activity (percentage of control)	
	Neurons from superior cervical ganglia	Neurons from ciliary ganglia
None	100	100
Heat	0	3
Dialysis	100	100
Trypsin	0	15
Trypsin plus Trasylol	100	100
Antiserum to NGF	100	100

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