Effects of Reversible Inactivation of the Primate Mesencephalic Reticular Formation. II. Hypometric Vertical Saccades

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INTRODUCTION

Recent data have demonstrated neurons with vertical on-directions located in the rostral portion of the mesencephalic reticular formation (MRF) adjacent to the interstitial nucleus of Cajal (INC) (Fukushima et al. 1990; Fukushima and Kaneko 1995; Handel and Glimcher 1997; Scudder et al. 1996; Silakov and Waitzman 1994). These MRF neurons had low-frequency long-lead burst activity that began 150 ms before vertical saccades (Handel and Glimcher 1997). A subsequent high-frequency burst began 8–32 ms before saccade onset (King et al. 1981; Scudder et al. 1996). Intracellular filling of these neurons has shown them to be located just lateral to the INC, dorsal-medial to the reticulotectal long-lead burst neurons (RTTLNs) (Scudder et al. 1996a), the focus of inactivation occurring in the previous paper (Waitzman et al. 2000). Cells in the peri-INC MRF (piMRF) region have axons that descend toward the pons and innervate the raphe nuclei (raphe pontis (RP), nucleus raphe interpositus (RIP), raphe obscuris (RO)) and the medullary reticular formation (primarily the inhibitory burst neuron region caudal to the abducens nucleus) (Scudder et al. 1996a). This pattern of arborization is distinct from the cells located in the INC per se. Cells within the INC project primarily to the oculomotor and trochlear nuclei and provide only a small projection to the spinal cord. The primary afferents to the INC arise from the rostral interstitial nucleus of the medial longitudinal fasciculus (riMLF). To the contrary, the piMRF receives direct projections from the superior colliculus (SC; primarily vertical and caudal regions) and provides strong innervation to the cervical spinal cord (Chen and May 2000; Kokkoroyannis et al. 1996; May et al. 1997). Another interesting aspect of these neurons is that they do not project back to the SC as do the RTTLNs in the more caudal portion of the MRF (see Waitzman et al. 2000). Thus cells in the piMRF are positioned both anatomically and physiologically to provide a vertical eye and possibly head (i.e., gaze) signal directly to the cervical spinal cord and to the cerebellum via projections to the nucleus raphe pontis.

Unilateral inactivation of the INC with muscimol produces an almost immediate loss of vertical gaze holding characterized by position-dependent, vertical, postsaccadic drift and a contralateral head tilt (Crawford and Vilis 1993). Unilateral loss or inactivation of the riMLF (i.e., the site of the medium lead vertical burst neurons) causes a reduction in downward eye velocity and loss of ipsitorsional saccades, whereas bilateral destruction eliminates all saccades in the vertical plane (Suzuki et al. 1995). We show here that unilateral inactivation of the piMRF produces a striking reduction in the gain of both up and down eye movements similar to the effect of bilateral riMLF lesions. Saccade duration was also prolonged. At the same time, position-dependent, vertical postsaccadic drift had not developed when saccade hypometria was evident. Simulations using a one-dimensional eye displacement model (see Waitzman et al. 2000, and Fig. 7B) suggested three possible explanations for saccade hypometria: 1) a shift in the input to the local feedback loop from a region coding for vertical
small eye displacement (Hypo #1), 2) combined reduction in the output of long-lead burst neurons (LLBNs) to vertical medium lead burst neurons (MLBNs) and shortened saccade triggering (Hypo #2), and 3) increased gain in the feedback path (Hypo #3). Only hypothesis #2 was able to reproduce saccade hypometria and increased saccade duration. The oculomotor (i.e., hypometric vertical saccades with longer durations) and postural difficulties of monkeys following piMRF inactivation closely parallel those seen in patients with progressive supranuclear palsy (PSP). These findings suggest that the bilateral input of the piMRF to nucleus reticularis pontis oralis (NRPo) and RIP is critical for the generation of accurate, rapid, vertical saccades. Abstracts of this work have appeared recently (Waitzman et al. 1997a,b).

METHODS

The methods for recording eye movements and single neurons, electrical microstimulation, and performing muscimol injections in three monkeys (G, C, and S) were the same as those described in detail in the accompanying paper (Waitzman et al. 2000).

Data analysis

A major analytic tool employed here was calculation of postsaccadic drift. This analysis was critical to our hypothesis that the piMRF and not the INC or riMLF was responsible for the vertical saccade hypometria reported here. The importance of the drift analysis is reflected by the use of two different methods used to calculate post-saccadic drift. As per the recommendation of Crawford and Vilis (1993) “drift amplitude,” the amplitude of drift following each saccade, is a very sensitive measure of integrator failure. These changes were noted within a few minutes of muscimol injection in the INC (Crawford and Vilis 1993). First, we calculated when significant drift amplitude appeared. This was done by comparing control drift amplitude ($A$, Crawford and Vilis 1993). First, we calculated when significant drift amplitude appeared. This was done by comparing control drift amplitude ($A$, Crawford and Vilis 1993) to drift amplitude calculated from $A$ (C–E), and $A$ (Table 1). Rostral muscimol injections

<table>
<thead>
<tr>
<th>Injection</th>
<th>Amount, $\mu$L</th>
<th>Concentration, $\mu$g/$\mu$L</th>
<th>Side</th>
<th>Head Tilt</th>
<th>Horizontal Drift Onset</th>
<th>Vertical Drift Onset</th>
<th>Onset of Hypometria</th>
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<td>70 min, $P &lt; 0.05$</td>
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Muscimol injections made in the piMRF of 3 monkeys. Lines in the injection column without labels indicate a repeated dose of the given amount at the same injection site.

RESULTS

Effects of muscimol: area of inactivation

Six injections of the GABA_A agonist muscimol were made in three monkeys (G, C, and S) at sites in the rostral MRF where vertical eye movement related cells were located (Table 1). All but two injections produced the same constellation of findings: 1) hypometria of both up and down saccades, 2) shift in the initial position of the eyes to the ipsiversive side and down, 3) curvature of saccade trajectories toward the horizontal or a plane tilted ~22° from the horizontal, 4) the duration of vertical saccades was prolonged and a small horizontal component appeared in five of six injections, and 5) a contra-versive head tilt. In two of six injections, the latency of vertical saccades was moderately shortened. Vertical post-saccadic drift was carefully monitored to determine when the INC was inactivated by spread of the muscimol (Table 1). Data were collected until the monkey could no longer perform the visually guided saccade paradigm.

Inactivation of the rostral MRF: vertical hypometria

Muscimol injection into the left MRF rostral to the posterior commissure and lateral to the INC produced the rapid onset of vertical saccade hypometria (Fig. 1). Quickly (within 10–25 min) upward (45° and 90°, positions 1 and 2) and downward saccades (270° and 315°, positions 6 and 7) became hypometric (Fig. 1, C–E). The progressive effect of muscimol over time was reflected by the increasingly negative difference coeffi-
FIG. 1. Hypometric vertical saccades developed after a muscimol injection in the rostral mesencephalic reticular formation (MRF) adjacent to the interstitial nucleus of Cajal (g021494). A: control trajectories of 25° visually guided saccades (VGS) to each of 8 different target directions. Zero degrees is to the right. B: 25° VGS trajectories 10 min after muscimol injection. Note the ipsiversive and downward displacement of initial eye position. C–E: “circle” plots showing amplitude of 25, 20, and 15° saccades 10–25 min after the injection. ○, average amplitude of at least 5 control saccades; ●, average amplitude of at least 3 postinjection saccades to each of 8 target positions. All initial positions are plotted at the origin. Times indicate the onset of eye movement recording after muscimol injection. Note the symmetrical rapid reduction in the amplitude of both up and downward movements of 15°. F: difference coefficients (ratio of post to preinjection amplitude, see METHODS) for the same saccades shown in C–E. Note the 20% reduction in saccade gain for 25° upward saccades at 5 min. At 25 min, there was an almost 50% reduction in the amplitude of upward and downward movements of 15°. G: saccade deviation plot shows that the endpoints of oblique and vertical saccades were deviated the most and were pushed closer to a plane tilted −22° from the horizontal. CCW, counterclockwise; CW, clockwise. H: muscimol injection (bottom filled star) was in the peri–interstitial nucleus of Cajal (INC) MRF −1.9 mm from the INC. A control saline injection was made just above this site (upper star). aq, aqueduct of Sylvius; BC, brachium conjunctivum; inc, interstitial nucleus of Cajal; III, oculomotor nucleus; ML, medial lemniscus; nuc. Interped., interpeduncular nucleus.
The metrics of the postinjection saccades were analyzed by comparing saccade amplitude, peak velocity, latency, and duration before and after the muscimol injections. The peak velocity of vertical saccades was reduced by >50% of the preinjection value following the muscimol injection (Fig. 2, B and D). One way the oculomotor system could compensate for the reduced vertical saccade amplitude would be to increase saccade duration and amplitude in the horizontal direction. Within 20 min after the muscimol injection, a small ipsiversive horizontal component of movement appeared in pure upward saccades (Figs. 1D and 2A, position 2). This was not evident for pure downward saccades (Fig. 2C).

The relationship between the peak velocity and saccade amplitude typically saturates for larger saccade amplitudes, the so-called main sequence (Fuchs 1967). The main sequence for preinjection control saccades was fit with a log regression (Fig. 2E, dotted line, $r^2 = 0.90$). Most postinjection saccades obeyed the main sequence. However, vertical saccades to positions 2 and 6 fell below the control regression (open circles and squares, respectively). Separate regressions were performed for the entire postinjection group of saccades (data not shown) and those to positions 2 and 6 (solid line). The latter were different from control ($P < 0.01$, Table 2). Thus the velocity of postinjection, vertical saccades was lower than expected for their vectorial amplitude despite the appearance of the small horizontal component of movement. This velocity reduction was evident for vertical saccades in the amplitude range of 7–25°.

Saccade latencies were unchanged after this injection despite separate analysis for pure vertical movements (Figs. 3A and 5C). On the other hand, the duration of postinjection vertical movements was longer than control after this injection (Fig. 3B). The duration of downward saccades (position 6) was slightly greater than upward movements (position 2), probably reflecting the appearance of a new horizontal component for upward and not downward saccades (compare Fig. 2, A with C, Fig. 3B, position 6 vs. position 2 regression). This stretching of the vertical saccade components and the addition of a new horizontal component contributed to the bending of saccades toward a plane tilted ~22° from the horizontal (Fig. 1D). Similar longer duration oblique saccades in response to vertically placed visual stimuli have also been observed in patients with PSP (Pierrot-Deseilligny et al. 1989; Rottach et al. 1996).

The appearance of position-dependent, postsaccadic drift after the injection heralded the spread of muscimol to involve nearby structures such as the INC. Typically there was little drift after each control saccade (Fig. 3, C and D, dotted regression lines, before injection). After the injection, drift amplitude remained within the ±95% confidence intervals of control up to 55 min for the horizontal channel and 30 min for the vertical channel (Fig. 3, C and D, open symbols). A relationship between postpseudacatic drift and eye position did not fully develop in either channel until 55 min (Fig. 3, C and D, closed symbols; see METHODS). Evidence of drift amplitude in the horizontal channel suggested that a torsional eye signal had appeared, possibly from INC inactivation.

### Summary of rostral MRF muscimol injections

Vertical saccade hypometria developed to varying degrees after all injections (Fig. 4). Increased saccade duration was noted in four injections (Fig. 5, A and B), and two injections showed a moderate reduction in saccade latency (Fig. 5C). Shifts in the initial position of the eyes were noted in five of six injections (Fig. 6B). The details are shown in the next three figures. All injections from the three monkeys within the pMRF region were projected onto a single section of the rostral midbrain (Fig. 4A). Two injections (c0430 and s0714) that were closer to the INC produced more upward than downward saccade hypometria (Fig. 4B, • and ▼). However, position-dependent, postpseudacatic drift did not appear

<table>
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### Changes in saccade metrics and trajectories that developed after the rostral MRF injection of Fig. 1. A–D: trajectories for horizontal (A and C) and vertical (B and D) components of 20° saccades 20 min after injection (data of Fig. 1D replotted). Saccade amplitude and velocity are plotted on the ordinate and time on the abscissa (right and up are positive). Dotted lines show control saccades, and solid lines show postinjection saccades. Note the marked reduction in amplitude and velocity of both upward and downward movements while saccade duration was increased (B and D). E: main sequence of vectorial amplitude vs. velocity for saccades before and after the same muscimol injection as in Fig. 1. The preinjection control data for all positions is shown by the dotted line. Controls for positions 2 and 6 are shown by a dashed line. Postinjection saccades to the 6 different target positions (0, 1, 3, 4, 5, and 7) are shown by different symbols. Saccades to position 2 (•) and position 6 (▼) fell below the control main sequence. Data from these 2 groups of postinjection saccades were fit with a separate regression (—). Both were different from control at the $P < 0.01$ level. The $r^2$ values for the fits were [control (positions 2 and 6): 0.90; exp (positions 2 and 6): 0.86].

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[Figure 2. Changes in saccade metrics and trajectories that developed after the rostral MRF injection of Fig. 1. A–D: trajectories for horizontal (A and C) and vertical (B and D) components of 20° saccades 20 min after injection (data of Fig. 1D replotted). Saccade amplitude and velocity are plotted on the ordinate and time on the abscissa (right and up are positive). Dotted lines show control saccades, and solid lines show postinjection saccades. Note the marked reduction in amplitude and velocity of both upward and downward movements while saccade duration was increased (B and D). E: main sequence of vectorial amplitude vs. velocity for saccades before and after the same muscimol injection as in Fig. 1. The preinjection control data for all positions is shown by the dotted line. Controls for positions 2 and 6 are shown by a dashed line. Postinjection saccades to the 6 different target positions (0, 1, 3, 4, 5, and 7) are shown by different symbols. Saccades to position 2 (•) and position 6 (▼) fell below the control main sequence. Data from these 2 groups of postinjection saccades were fit with a separate regression (—). Both were different from control at the $P < 0.01$ level. The $r^2$ values for the fits were [control (positions 2 and 6): 0.90; exp (positions 2 and 6): 0.86].]
until >20–30 min after the onset of vertical saccade hypometria (Table 1). This suggested that the reduction in upward saccade amplitude occurred first and then the muscimol spread to inactivate a portion of the INC. Vertical hypometria ranged from 80% to almost 40% of preinjection vertical saccade amplitude (Fig. 4B). Note that these numbers are underestimates because we have shown in Fig. 4B the time point at which the monkey could still make saccades in all directions.

In four of six injections, saccade duration of the vertical component of eye movement was markedly prolonged (Fig. 5B). A new horizontal saccade component appeared in five of six injections (Fig. 5A). Last, the latency of vertical (position 2 and 6) saccade onset following four of six injections was modestly shorter and reached significance in two experiments (Fig. 5C).

**Effects on spontaneous saccades**

After each of the injections, a file of spontaneous saccades made in total darkness was collected. In contrast to the effects found in the accompanying paper (Waitzman et al. 2000), no specific goal of eye movement was noted (Fig. 6A). The average

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**FIG. 3.** Effects of the muscimol injection of Fig. 1 on saccade latency, duration, and postsaccadic drift. A: latency was unchanged after this muscimol injection. ●, latency of postinjection saccades to positions 2 and 6. Dotted regression line shows control latency. B: saccade duration was increased after this injection particularly for vertical saccade components. Duration of the vertical component was plotted against vectorial amplitude as per King et al. (1986). C and D: postsaccadic drift (i.e., drift amplitude) for both the horizontal (C) and vertical (D) channels are shown as a function of postsaccadic vertical eye position (abscissa). Open symbols represent time points obtained before significant drift amplitude developed (see METHODS). Filled symbols represent drift amplitude after the onset of postsaccadic drift, suggesting that the INC was inactivated. Separate regressions were fit to the pre- (Horizontal $r^2 = 0.0009, m = -0.001; \text{Vertical } r^2 = 0.24, m = -0.03$) and postsaccadic drift data (Horizontal $r^2 = 0.14, m = -0.14; \text{Vertical } r^2 = 0.21, m = -0.22$).
locus of the endpoints for all spontaneous saccades is shown in Fig. 6A. The monkey made saccades toward the central fixation point. In one injection (s0714) the average position shifted in the same direction as the shift in initial eye position.

Head tilt and shift in initial position

Inactivation of the INC with muscimol has been reported to produce a position-dependent, postsaccadic drift and a contralateral head-tilt in monkeys (Crawford and Vilis 1992; Fukushima et al. 1987). Five of the six rostral injections reported here produced position-dependent, postsaccadic drift within 70 min of initiation of injection (Table 1). However, within 10–20 min all but one of the rostral MRF muscimol injections demonstrated an ipsilateral and downward shift in the initial position of the eyes (Fig. 6B). As demonstrated in the head-free monkey (see accompanying report, Waitzman et al. 2000), an ipsilateral shift in the initial position of the eyes in the head-fixed monkey could in fact be compensatory for an attempted contralateral head tilt in the head-free monkey. This compensation brought gaze (combined head and eye signal) close to zero (Waitzman et al. 2000). Three of six rostral injections produced a contralateral head tilt of 20–30° after the monkey’s head was released from the head fixation device 2–3 h after the injection. No experiment was performed during which the head was released immediately after a muscimol injection in the rostral MRF. Postural abnormalities were also observed and included an inability to maintain an upright posture after the injection without hemiparesis or sensory loss. Gait and posture returned to normal within the next 24 h.
DISCUSSION

The results presented here support the idea that the MRF is divided into at least two portions. Inactivation of the ventral-caudal MRF, which corresponds to nucleus subcuneiformis, produced saccade hypermetria (Waitzman et al. 2000). Single cell recordings in the rostral MRF, which we designate the

![Graph A](image)

**A**  Vectorial Amplitude vs Horizontal Duration (Positions 2 & 6)

![Graph B](image)

**B**  Vectorial Amplitude vs Vertical Duration (Positions 2 & 6)

![Graph C](image)

**C**  Average Vectorial Latency for Rostral Muscimol Injections for Positions 2 & 6

FIG. 5. Changes in the amplitude/duration relationship and saccade latency following injections in the piMRF. *A* and *B*: horizontal (*A*) and vertical (*B*) component saccade duration was plotted against vectorial amplitude for saccades to target positions 2 and 6 before and after muscimol injection (King et al. 1986). Slopes of the linear regression of postinjection are compared with preinjection, control vertical saccades (*B*, open bars = control). Note that horizontal saccade duration was zero for preinjection vertical saccade and thus no control is shown. *C*: the latency for saccades to vertical target positions (2 and 6) were measured before (open bars) and after (hatched bars) injection. Those injections that achieved significance are indicated by asterisks above the bars (see key).
lesions there was an \(~50\%\) reduction in the amplitude of downward saccades. A similar reduction has also been observed after muscimol injections in the INC (Crawford and Vilis 1993). We compared results of riMLF lesions to the inactivation of the piMRF by performing a curve fit of the saccade velocities of the Suzuki et al. (1995) data and derived saccade amplitude by integration (results not shown). The amplitude of upward saccades was normal, whereas that of downward saccades was reduced by 30\% and duration was modestly increased (Suzuki et al. 1995). These effects on downward saccades following riMLF lesions are quite different from the 50\% reduction of vertical saccade amplitude noted following the current muscimol injections into the piMRF (e.g., Fig. 1).

Three pieces of evidence eliminate the possibility that the vertical hypometria demonstrated here was the result of inactivation of the INC. First, both up and down saccades [except for s0714 and c0430, Fig. 4 (summary), which initially affected upward saccades only] quickly became hypometric. This would be atypical for INC inactivation because it affected downward saccades to a greater degree than upward saccades (Fukushima and Kaneko 1995; Helmchen et al. 1998). Second, the reduction in vertical saccade amplitude described previously after INC inactivation occurred after bilateral, not unilateral inactivation. Third, all reported INC inactivations were associated with difficulty with vertical saccade holding, i.e., position-dependent, postsaccadic drift. In all six peri-INC MRF injections, vertical saccade hypometria occurred before the development of position-dependent, postsaccadic drift (Table 1, Fig. 3). Taken together, the above analysis provides strong evidence that a critical region of the rostral MRF, lateral to the INC and riMLF and rostral to the posterior commissure, was responsible for the vertical saccade hypometria. This area, as defined by the centers of all injection sites, has been projected onto a single rostral MRF section. It encompasses a region 2 mm wide, 2 mm deep, and 2.5 mm in rostral-caudal dimensions and is designated the peri-INC MRF (piMRF, Fig. 4A, dotted region).

Unilateral or bilateral muscimol injections

Before exploring the functions of the piMRF further, we provide a number of observations that suggest that all six muscimol injections were unilateral. First, the onset of vertical saccade hypometria was very rapid requiring \(<20\) min following rostral injections to become apparent. Second, the histological sections show that only one of the tracks (s0721) crossed the midline within the brain stem. Third, the rate of spread of the muscimol was directly measured in control experiments (see accompanying paper, Waitzman et al. 2000), and a spherical region of inactivation 2 mm in size was found 3 h after the injection. Inactivation of MLBNs in the riMLF produces downward saccade hypometria and an ipsiversional shift in Listing’s plane (Crawford 1994; Crawford and Vilis 1992, 1993; Helmchen et al. 1998; Suzuki et al. 1995; Vilis et al. 1989). Combined paralysis of upward and downward movements has not been observed following unilateral inactivation or destruction of riMLF (Crawford 1994; Crawford and Vilis 1992, 1993; Suzuki et al. 1995; Vilis et al. 1989). In a similar vein, inactivation of the INC quickly (within 15 min) generates a 50\% reduction in vertical eye velocity and typically position-
hypometria was evident 4–40 min after injection (Crawford 1994; Crawford and Vilis 1992, 1993; Helmchen et al. 1998; Suzuki et al. 1995; Vilis et al. 1989). In the current experiments, clear vertical saccade hypometria was evident 4–40 min before the appearance of vertical, post-saccadic drift (Table 1).

Last, after the end of each experiment, the monkey's head was released. In all experiments in which a prominent head tilt was observed, it was contraversive (to the right shoulder following a left side injection, Table 1) and not backward or to the ipsilateral side. This suggested that unilateral, not bilateral inactivation of the brain stem had occurred (Crawford 1994; Crawford and Vilis 1992, 1993; Fukushima et al. 1987). In sum, these results support the idea that unilateral inactivation of the rostral MRF led to rapid deficits in vertical saccades. This raises two questions regarding vertical eye movement organization. First, if the INC and/or the riMLF were not responsible for the observed changes in vertical saccades, how does inactivation of the piMRF lead to a vertical gaze palsy and prolonged duration of vertical saccades? Second, the prevailing dogma has stipulated that bilateral inactivation or destruction of the cerebral cortex or brain stem structures is required to produce significant impairment of vertical saccades (Christoff 1974; Christoff et al. 1962; Pasik et al. 1969). How can a unilateral inactivation of the rostral MRF with muscimol lead to effects on both up and down saccades?

Role of piMRF in oculomotor control: anatomic connections

The anatomic connections and previously described physiological characteristics of piMRF neurons (Handel and Glimcher 1997) suggest answers to both questions. Consider first saccade durations and bilateral riMLF inactivation. The MLBNs in the riMLF burst 20–30 ms before the onset of saccades with either upward, downward, or torsional components of movement (King and Fuchs 1977, 1979; King et al. 1981; Vilis et al. 1989). Despite the presence of both up and down bursters on each side of the brain stem, both riMLFs must be activated to produce purely vertical saccades (Bender and Shanzler 1964; Christoff et al. 1962). To generate horizontal saccades, a cascade of LBNNs in the SC and elsewhere eventually activate MLBNs in the paramedian zone of the pontine reticular formation (PrRF) (Hepp and Henn 1983; Moschovakis et al. 1996). A loss of these LLBNs will produce a reduction in the peak discharge of the burst in pontine reticular formation (prRF) and therefore a stretched (longer duration) horizontal component. We (Waitzman et al. 1997a) as well as others (King et al. 1981; Kokkoroyannis et al. 1996; Moschovakis et al. 1996) have proposed a similar physiological cascade for the vertical system. If this were so, then the muscimol injections would reduce the peak discharge of the LLBNs in the piMRF neurons and secondarily cause similar reductions in the discharge frequency of the MLBNs in the riMLF bilaterally. This would lead to prolonged but accurate vertical saccade components. Despite the simplicity of this idea, direct connections between the peri-INC LBNNs and the riMLF have not been demonstrated (Buttner-Ennever and Buttner 1988; Kokkoroyannis et al. 1996; Robinson et al. 1994).

At least three indirect, alternative paths exist that could carry LLBN activity from the piMRF to MLBNs in the riMLF (Fig. 7A). A disynaptic pathway from the piMRF to the SC and then to the riMLF (Chen and May 2000; May et al. 1997) is the fastest route for modulation of riMLF neurons by MRF LLBNs (pathway 1, Fig. 7A). These connections have been confirmed in both the cat (Nakano et al. 1985) and the monkey (Kokkoroyannis et al. 1996). A second pathway that may include longer disynaptic or multisynaptic delays could be mediated via bilateral projections from the peri-INC LBNNs to NRPo of the pontine reticular formation (location of pontine LLBNs) as well as to the raphe interpositus (rip; omnipause neurons; pathway 2) (Buttner-Ennever, personal communication). Both of these regions provide dense descending projections to the riMLF (Nakano et al. 1985; Scudder et al. 1996). A third route for modulation of the riMLF output could be mediated via a piMRF → cerebellum → SC loop (Lefevre et al. 1998). Cells in the piMRF provide descending projections to RP, RO, and bilaterally to the inhibitory burst neuron region of the medullary reticular formation just caudal to the PPRF (IBNm-RF; pathway 3). Raphe pontis and obscuris also project to lobule VII and the flocculus of the cerebellum and are known to carry saccade-related signals (Blanks and Precht 1983; Blanks et al. 1983; Langer et al. 1985; Nakano et al. 1985; Scudder et al. 1996b). The flocculus is also important in gaze holding and is critical in the adaptive control of post-saccadic drift (Optican et al. 1986; Zee et al. 1981). The primary cerebellar input to the SC arises from the fastigial nuclei that receive a strong projection from the flocculus and the cerebellar vermis, lobules VI and VII. Again like pathway 1, the SC would mediate activity in this path. Seen in this light, the role for the bilateral projections from the piMRF to NRPo, medullary reticular formation, and raphe interpositus (rip; pathway 2) is accentuated because it provides a route for the generation of spontaneous saccades without the SC acting as an intermediary. It is the loss of such bilateral inputs that would be expected to produce the longer saccade durations observed after piMRF inactivation.

The answer to the question of reduced saccade amplitude is less clear. As noted earlier, changes in the input to the local feedback loop could produce saccade hypometria. This could arise via the pathway from the piMRF to the SC (pathway 1, Fig. 7A). Thus a shift in activity from a caudal to a more rostral portion of the SC could produce hypometric vertical saccades with normal duration. Changes within the feedback pathway while affecting saccade dynamics most often produce minimal reduction in saccade amplitude, the controlled variable. As a result, reduced LLBN output to MLBNs in the riMLF would yield longer duration, normal amplitude vertical saccades. However, if reduced LLBN output were coupled to changes in the triggering mechanism of saccades, significant reduction in saccade amplitude could result. For instance, suppose the omnipause neurons could more easily flip on from off and vice versa. This would lead to shorter saccade latency, which was observed in four injections (Fig. 5C). In addition, the tendency to return to a tonic firing level would also increase. Reactivation of omnipause neurons is dependent on how close the saccade is to target (i.e., residual motor error). If reactivation occurred at larger residual error, saccades would be shorter because the pause would be truncated before the plant reached the target. Timed correctly, this could account for vertical saccades that fall short of their goal despite longer durations. Such effects could be mediated via pathway 2 leading from the piMRF to RIP and NRPo. Recording of activity in the NRPo
and rip during piMRF inactivation would permit these ideas to be tested physiologically.

Role of the piMRF in oculomotor control: models

In addition to physiology and anatomy, simulation of the one-dimensional eye displacement model (retinotopic coordinates) described previously was used to examine these hypotheses (Jurgens et al. 1981; Waitzman et al. 1996; see also accompanying paper, Waitzman et al. 2000) (Fig. 7B). As noted above, reduction in the amplitude of vertical saccades could occur in three different ways. Either the desired change in eye displacement (ΔE, the input to local feedback loop) could be reduced (Hypothesis #1), the input to the MLBNs in the riMLF could be reduced in combination with changes in saccade triggering (Hypothesis #2), or feedback gain could be increased (Hypothesis #3). Changes in the input to the feedback loop (Hypothesis #1) could occur by shifting activity in the SC from a region coding large amplitude to a nearby region coding small saccades. Typically, changes within the local feedback loop do not affect the amplitude of saccades. However, we provide two different “within-loop” scenarios that generate saccade hypometria, and the resultant saccades have very different dynamics. Hypothesis #2 suggests that reduction of the motor error signal input (presumably from reduced LLBN output) to the MLBNs is coupled to early reactivation of the omnipause neurons (i.e., removal of the trigger) before the saccade reached the target. In this scheme, saccade duration would most likely be lengthened because the eyes were moving more slowly (reduced MLBN input). However, saccade amplitude would be inaccurate because the omnipause neurons were reactivated before the eyes reached the target. Long duration, but accurate horizontal saccades have been observed clinically following damage to the cerebellum (Zee et al. 1975). In this situation, the omnipause neurons were held off until motor error went to zero. In Hypothesis #3, increased gain would produce a series of smaller, but short duration saccades that eventually brought the eyes onto target.

The results of simulating these ideas are shown in Fig. 8.
A  Hypometria #1: Shifting the Input  
Hypometria #3: Increased Feedback Gain

B

Time (s)

Velocity (deg/s)

0 200 400 600

0 0.20 0.25

C  Hypometria #2: Reduced input to Burst Generator

D

Time (s)

Velocity (deg/s)

0 200 400 600

0 0.20 0.25

E

Time (s)

Amplitude (deg)

0 5 10 15

0 0.0 0.5 1.0

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50% reduction in the desired change in position (ΔE) resulted in a 50% smaller, but normally executed saccade (i.e., Hypo #1, normal saccade dynamics, Figs. 7B and 8, A and B, — —). Within the feedback loop the motor error input to the MLBNs of the riMLF was reduced by 80%, and the omnipause neurons (i.e., the trigger) were reactivated when the saccade reached within 1° (previously 0.1°) of the proscribed motor error (Hypo #2, Fig. 7B). This produced a 60% reduction in saccade amplitude and a 20% increase in saccade duration (Fig. 8, C and D, — —). Desired change in eye position (the input to the local feedback loop) remained at its original value, leaving an unresolved motor error. This generated a staircase of saccades that brought the eyes onto target (Fig. 8E). This scenario corresponds closely with the results of the injections shown here. Increasing the gain of the feedback pathway by 50% (Hypo #3, Fig. 7B) produced a 50% reduction in the amplitude of the initial saccade (Fig. 8, A and B). Again a staircase of smaller saccades was generated to bring the eyes eventually onto target (Fig. 8E). However, each saccade of the staircase was of shorter duration and slightly higher velocity than a saccade executed without the change in the gain of the feedback pathway (Fig. 8, A and B, — - - — — — —, respectively). Shortened saccade duration was evident following one piMRF inactivation (Fig. 5B, s0714). Possibly this injection could have changed feedback gain. On the other hand, four of six piMRF injections produced hypometric, longer duration saccades. This suggested that hypothesis #2, reduced LLBN activity coupled with changes in saccade triggering, could best account for our observations.

MRF: participation in head control

Two findings in the current study extend the results of the previous paper (Waitzman et al. 2000), suggesting a strategic role of the MRF in the control of head movement and posture. First, three of six injections resulted in a contralateral head tilt and postural instability (Table 1), albeit observed 2–3 h after the muscimol injection. At the same time, there was a clear offset of the initial position of the eyes to the ipsilateral side within 20 min of all rostral injections (including that of g021494). We suggest that this ipsilateral and often downward shift in eye position would almost exactly compensate for a contralateral and upward head tilt. A similar ipsilateral offset of primary position was noted in the previous paper with the onset of a contralateral head tilt in the head-free monkey (Fig. 14 in Waitzman et al. 2000). Taken together these observations suggest that inactivation of the peri-INC MRF produced a compensatory ipsilateral shift in primary eye position before the onset of inactivation of the INC.

Some controversy surrounds the exact role of the INC in head control (Crawford 1994; Crawford and Vilis 1992, 1993; Crawford et al. 1991; Fukushima 1987; Fukushima et al. 1987). A number of studies agree that there are ipsilateral descending connections from the INC to the vestibular nuclei (Fukushima et al. 1987; Kokkoroyannis et al. 1996; Robinson et al. 1994). However, there is disagreement regarding the direct projections from the INC to the cervical spinal cord. One study using retrograde transport from the cervical segments suggested a sparse descending projection from the INC (Robinson et al. 1994). A more recent study using intracellular recording and biocytin neuroanatomy demonstrated that INC neurons of the squirrel monkey do terminate in the ipsilateral ventral horn of the upper cervical segments (Kokkoroyannis et al. 1996). However, all studies demonstrate much larger ipsilateral projections from the cMRF, peri-INC MRF, and cuneate reticular nucleus to the upper cervical cord than from the INC (Castiglioni et al. 1978; Kokkoroyannis et al. 1996; Robinson et al. 1994; Seudder et al. 1996a). This suggests that lesions and reversible inactivation that have been directed at the INC in the past may in fact have inhibited or damaged both the piMRF and the INC itself. Therefore both the MRF and the INC may be important sources of descending midbrain activity to the cervical cord related to the head and postural control and may account for the contralateral head tilt and postural abnormalities noted following rostral MRF injections (Fukushima 1987; Kokkoroyannis et al. 1996; Robinson et al. 1994).

Relationship to mesencephalic disorders of eye movement

PSP is a degenerative neurological disorder in which neurofibrillary tangles are deposited in the reticular formation and basal ganglia ( Steele et al. 1964). These patients exhibit difficulties with balance, neck movement, and a progressive restriction in voluntary vertical rapid eye movements. Eventually patients also develop microsaccadic square-wave jerks and restricted horizontal eye movements (Fensore et al. 1988; Gomez et al. 1990; Hershkowitz et al. 1989; Rafal et al. 1988). Despite these deficits, conjugate eye movements (including quick phases of nystagmus) induced by vestibular activation remain intact (Perirot-Deseilligny et al. 1989; Rottach et al. 1996). Neuropathological changes target vertical and horizontal eye movement regions of the brain stem, including the riMLF, the SC, the PPRF, the MRF, but not the oculomotor nuclei (Juncos et al. 1991; Steele et al. 1964; Zweig et al. 1987).

However, destruction of the riMLF or PPRF cannot explain the clinical features of PSP, because the quick phases of vestibular and optokinetic nystagmus persist and are mediated by these same structures. Bilateral removal of the riMLF in both patients and nonhuman primates impairs all vertical rapid eye movements, i.e., both saccades and vestibularly induced quick phases (Vilis et al. 1989). Similarly, destruction of the
PPRF on one side abolishes ipsilateral, horizontal saccadic eye movements and the quick phases of vestibular nystagmus, whereas smooth pursuit and the slow component of vestibular nystagmus are preserved in the horizontal direction (Goebel et al. 1971; Henne et al. 1984). These lesion studies suggest that other pontomesencephalic areas are targeted to produce the characteristic oculomotor findings observed in patients with PSP.

One of the curious features of PSP is the appearance of vertical eye movement abnormalities initially that are followed by impairments in horizontal saccades and the generation of square-wave jerks. These characteristics are noted in the monkey experiments presented here and in the accompanying paper (Waitzman et al. 2000). Initially, the effects of rostral MRF inactivation were exclusively on vertical eye movements. However, horizontal deficits appeared as the musculomotor injection spread with an inability of the monkey to make contraversive saccades across the midline. Interestingly, as pointed out in the accompanying paper (Waitzman et al. 2000) macrorosaccadic square-wave jerks and a contraversive head tilt appeared quickly after many caudal injections (e.g., g0217 and c0521) (Waitzman et al. 2000). Thus the vertical hypometria of PSP patients could result from the loss of the more rostral MRF, whereas increased neck tone, imbalance, and square-wave jerks could be secondary to loss of more caudal portions of the MRF. Taken together, these two groups of injections suggest that patients with PSP could have neuropathological involvement that begins rostrally and then proceeds caudally within the MRF. Neuropathological study of patients with documented PSP at various stages of the illness could help determine whether such a hypothesis were true.

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