Effects of Reversible Inactivation of the Primate Mesencephalic Reticular Formation. I. Hypermetric Goal-Directed Saccades

DAVID M. WAITZMAN, VALENTINE L. SILAKOV, STACY DePALMA-BOWLES, AND AMANDA S. AYERS

Department of Neurology, The University of Connecticut Health Center, Farmington, Connecticut 06030

Effects of reversible inactivation of the primate mesencephalic reticular formation. I. Hypermetric goal-directed saccades. J. Neurophysiol. 83: 2260–2284, 2000. Single-neuron recording and electrical microstimulation suggest three roles for the mesencephalic reticular formation (MRF) in oculomotor control: 1) saccade triggering, 2) computation of the horizontal component of saccade amplitude (a feed-forward function), and 3) feedback of an eye velocity signal from the paramedian zone of the pontine reticular formation (PPRF) to higher structures. These ideas were tested using reversible inactivation of the MRF with pressure micro-injection of muscimol, a GABA<sub>A</sub> agonist, in four rhesus monkeys prepared for chronic single-neuron and eye movement recording. Reversible inactivation revealed two subregions of the MRF: ventral-caudal and rostral. The ventral-caudal region, which corresponds to the central MRF, the cMRF, or nucleus subcuneiformis, is the focus of this paper and is located lateral to the oculomotor nucleus and caudal to the posterior commissure (PC). Inactivation of the cMRF produced contraversive, upward saccade hypermetria. In three of eight injections, the velocity of hypermetric saccades was too fast for a given saccade amplitude, and saccade duration was shorter. The latency for initiation of most contraversive saccades was markedly reduced. Fixation was also destabilized with the development of macrosaccadic square-wave jerks that were directed toward a contraversive goal in the hypermetric direction. Spontaneous saccades collected in total darkness were also directed toward the same orbital goal, up and to the contraversive side. Three of eight muscimol injections were associated with a shift in the initial position of the eyes. A contralateral head tilt was also observed in 5 out of 8 caudal injections. All ventral-caudal injections with head tilt showed no evidence of vertical post-saccadic drift. This suggested that the observed changes in head movement and posture resulted from inactivation of the caudal MRF and not spread of the muscimol to the interstitial nucleus of Cajal (INC). Evidence of hypermetria strongly supports the idea that the ventral-caudal MRF participates in the feedback control of saccade accuracy. However, development of goal-directed eye movements, as well as a shift in the initial position following some of the cMRF injections, suggests that this region also contributes to the generation of an estimate of target or eye position coded in craniotopic coordinates. Last, the observed reduction in contraversive saccade latency and development of macrosaccadic square-wave jerks supports a role of the MRF in saccade triggering.

INTRODUCTION

Three possible oculomotor roles have been suggested for the central mesencephalic reticular formation (cMRF) (Waitzman et al. 1996). Subthreshold low-frequency electrical microstimulation and single neuron recording of a low-frequency, long-latency (15–100 ms) discharge before saccades support the idea that the cMRF participates in saccade triggering (Cohen et al. 1985, 1986; Handel and Glimcher 1997; Waitzman 1982, 1992; Waitzman et al. 1996). Second, existence of cMRF neurons with contralateral movement fields that increase their discharge with the horizontal but not the vertical component of movement suggests that these cells could serve as a spatial filter extracting the horizontal component of movements from the superior colliculus (SC) output (Sparks 1986; Sparks and Mays 1990; Waitzman et al. 1996). Cells in the rostral portion of the MRF (see accompanying paper) may participate in the generation of the vertical component of saccadic eye movement (Handel and Glimcher 1997). Third, by virtue of a burst of activity that peaks just before and during saccades and dynamics of the neural discharge that correlate closely with either eye velocity and/or displacement, we have hypothesized that cMRF neurons could participate in the feedback control of saccades (Waitzman et al. 1996).

The impact of each of these hypotheses on saccade generation is illustrated with the help of two feedback models of oculomotor control (Fig. 1). The eye position model shown in Fig. 1A was based on the original, local-feedback model of Robinson (1975). Current eye position in space (Eye) was subtracted from target position in space (Targ) by the retina, to produce a retinal error signal (R<sub>err</sub>). Robinson’s major contribution was to suggest that retinal error was added to an internal copy of eye position (i.e., efference copy, or corollary discharge, E’) in craniotopic coordinates to create an estimate of target position with respect to the head not the retina (Targ<sub>es</sub>). In a subsequent step, efference copy (E’) was subtracted from a delayed copy of target position to generate a motor error signal (e<sub>m</sub>) used to drive the burst neurons in the pontine reticular formation (B). Integration of the velocity output of the burst neurons (V<sub>b</sub>) by the neural integrator (NI) produced an eye position signal used to drive the ocular motoneurons. Two unique properties emerged from this model. First, by virtue of local feedback, burst output continued for as long as necessary to get the eyes onto the target and explained many aspects of the relationship between saccade amplitude and duration. Second, the input to the oculomotor system was a target position with respect to the head signal. This property in particular made it easy to incorporate vestibular inputs (Robinson 1975). However, since its proposal, a number of objections have been raised to this model and question its applicability to the ocu-
One primary concern has been that few regions of the brain contain eye position activity [i.e., nucleus prepositus hypoglossi (NPH) and the interstitial nucleus of Cajal (INC)]. More importantly, these regions do not project back to areas such as the SC, which should receive feedback of this efference copy of eye position.

The eye displacement model shown in Fig. 1B addressed these issues by placing a resettable integrator (RI) into the local feedback pathway. This modification transformed the inputs of the model into retinotopic coordinates (Jurgens et al. 1981; see Waitzman et al. 1991, 1996 for further discussion of this model). Briefly, the input to the model, desired eye displacement (ΔE), was thought to arise from the frontal eye fields (FEF) and dorsomedial frontal cortex (DMFC). The desired displacement was compared with current eye position (e_m) to produce a motor error (e_m). Motor error, which represents how far the eyes must move to point toward the target, drives the burst neurons in the pontine reticular formation (PPRF) through a switch (trigger) thought to represent the omnipause neurons. The “velocity command” [V_c; output of the medium lead burst neurons (MLBs)], B, is applied to the neural integrator (NI) to generate current eye position (an internal representation of eye position) and to the oculomotor plant to produce the actual eye movement. Reductions in desired eye position will lead to hypometric saccades, whereas interruption in the generation of the current eye position (i.e., loss of feedback) will generate hypermetric saccades. B: the local feedback portion of the above model (dashed rectangle) is recast into “change in eye position” coordinates (Jurgens and Becker 1981). The input to the local feedback loop is now a desired eye displacement (ΔE) that is compared with current eye displacement to generate motor error (e_m). Again e_m drives the MLBs through a switch. However, now the output of the MLBs, V_c, is fed back through a resettable integrator (RI) to generate current eye displacement (ΔE'). V_c is also applied to the NI to hold the eyes steady at a new location following each saccade, eye position (E'). This diagram illustrates that an increase in the input (ΔE) to the feedback loop can lead to saccade hypermetria (Hyper #1). Damage to the RI or feedback path itself could also lead to saccade hypermetria (Hyper #2). Reduction in the trigger threshold will initiate saccades earlier (i.e., reduced saccade latency). Increased delays in the 2nd feedback pathway (Hyper #3) will lead to macrosaccadic square-wave jerks and in some instances, hypermetria (see Discussion).
PPRF. The output of the burst neurons was a velocity command \( \dot{V}_s \) that was directed to both the NI and a RI \( \Delta E' \) or the efference copy whose output was reset to zero at the end of each saccade. The purpose of the NI was to hold the eyes steady following the occurrence of each saccade while the output of the RI was used to update higher structures of the current displacement of the eyes. The NI for the horizontal saccade component is generated in the NPH (Cannon and Robinson 1987), and the NI for the vertical component of saccades is thought to originate from the INC (Crawford et al. 1991). The source of the trigger signal used to initiate saccades is thought to be the omnipause neurons located in the nucleus raphe interpositus (RIP).

Predictions about the specific oculomotor deficits, which may occur after inactivation of brain stem structures, are easier to understand by reference to these models. Shifts in the input to either model, that is a more distant orbital position (EP model), or larger eye displacement (ED model), would result in saccades that overshoot the goal (Fig. 1, A and B, Hyper #1). A shift in input could occur if cMRF neurons performed a spatial filter role for the SC and FEF output (Sparks 1986; Sparks and May 1990; Waitzman et al. 1996). Simulations of these various aspects of the models are presented in the DISCUSSION.

Reduction or damage to the pathways within the feedback loop would eventually produce a reduction in either the current eye position (EP model) or eye displacement (ED model) feedback signals. This reduction would increase the duration of the motor error signal \( e_m \), and the eyes would continue to move beyond their goal, albeit at a slower velocity (Fig. 1, A and B, Hyper #2). Thus, in the ED model if the reticulotectal, long lead burst neurons (RTLLBNs) of the cMRF provide a conduit for a velocity signal from the PPRF to the SC, or participate in the process of integrating eye velocity (i.e., the RI), loss of these cells should produce saccade hypermetria. This result would correlate well with the feedback hypothesis (Waitzman et al. 1996). However, damage to the feedback mechanism of the ED model could not produce a change in initial eye position or generate a saccade goal.

Reduction of feedback or damage to the neural integrator itself in the EP model would also produce hypermetric, slow saccades (Fig. 1A, Hyper #2). However, in this instance, shifts in initial position and generation of a saccade goal relative to the head could result. Moreover, damage in the second portion of the feedback pathway of the EP model (Fig. 1A, Hyper #3) could increase delays in the generation of the \( \text{Tar}_{eq} \) and cause repeated saccades to a virtual target that continues to reappear (see DISCUSSION). Finally, making the saccade trigger easier to flip from opened to closed and vice versa could make saccade latency shorter. This might occur if excitatory activity from cMRF neurons important for maintaining the tonic firing of omnipause neurons was removed (i.e., the triggering hypothesis) (Cohen et al. 1985; Hepp and Henn 1982, 1983; Waitzman et al. 1996). Providing clear neurophysiological evidence to support each of these hypotheses of MRF participation in oculomotor control has proven difficult. The midbrain tegmentum contains both cells and fibers in passage from the superior colliculus and other structures. As a result, the destruction or activation of the collicular output may have biased previous electrolytic lesion and electrical microstimulation experiments (Cohen et al. 1982, 1985, 1986; Komatsuzaki et al. 1972).

The current group of experiments has been designed to circumvent some of these difficulties. Following electrical microstimulation and single and multiunit identification of the MRF, we have made microinjections of muscimol, a GABA\(_A\) agonist. We demonstrate that the MRF can be divided into two separate regions. Inactivation of a ventral-caudal region, which corresponds to the nucleus subcuneiformis (the cMRF), leads to oblique (contraversive and up) saccade hypermetria, higher saccade velocity, reduced saccade duration, and marked instability in fixation with the development of macrosaccadic square-wave jerks to a specific goal in the orbit (the current paper). Inactivation of the rostral portion of the MRF results in severe hypometria primarily of vertical, but not horizontal saccades (see accompanying paper, Waitzman et al. 2000). The implications of these findings are discussed with reference to the two models and three possible hypotheses for cMRF function just presented. Abstracts of these findings have appeared previously (Silakov and Waitzman 1996; Waitzman and Silakov 1994; Waitzman et al. 1997).

METHODOLOGY

The methods for recording eye movements and single neurons, electrical microstimulation, and data analysis in awake behaving primates in these experiments are essentially the same as those described in detail elsewhere (Waitzman et al. 1991, 1996). All procedures were approved by the University Animal Care and Use Committee.

Injection and recording procedures

In brief, four male rhesus monkeys (\( G, C, K, \) and \( T \)) were surgically prepared under isoflurane inhalational anesthesia with two eye coils (Judge et al. 1980), a head restraining device, and two stainless steel chambers to allow separate access to the MRF and the SC. The MRF cylinder was positioned over the posterior portion of the cerebral cortex tilted 15° off the sagittal plane (Waitzman et al. 1996). The MRF, located just lateral to the oculomotor nuclei, was identified by the characteristic features of single neurons that discharge with contraversive saccadic eye movements and electrical microstimulation that elicited contraversive, conjugate saccades at short latency (Silakov et al. 1995; Waitzman et al. 1996). Eye movements were recorded using the magnetic search coil technique and were accurate to 0.1° (Judge et al. 1980). In two monkeys, a series of guide tubes were placed parallel to each other and sampled the rostral, mid, and caudal portions of the MRF. The tubes were semipermanently positioned using a grid (spacing of 1 mm) fixed within the stainless steel recording chamber. In the third and fourth monkeys, only the caudal portion of the MRF was sampled. The arrangement of a rostral-caudal orientation of the guide tubes allowed for repeated testing and subsequent permanent identification of the sites of muscimol injections. A customized microinjection/recording needle (Crist et al. 1988) attached to a Hamilton syringe allowed for physiological confirmation of neuronal activity related to saccades before an injection and monitoring of neuronal activity after the injection. In monkey \( T \), a picospritzer apparatus was substituted for the Hamilton syringe (Dias and Segraves 1997).

Behavioral paradigms

Figure 2A shows the fixation paradigm, and Fig. 2, B–D, illustrates the visually guided saccade (VGS) paradigm. Visual targets were positioned at eight different directions [0° (position 0), 45° (1), 90° (2), 135° (3), 180° (4), 225° (5), 270° (6), and 315° and/or 45° (7)] and five amplitudes (5, 10, 15, 20, and 25°) along each of these
directions for a total of 40 different target locations. The monkey was trained to fixate a central light-emitting diode. After a variable interval of 200–400 ms, the light was extinguished, and a new target light appeared that was the cue for the monkey to shift his eyes and fixate the new visual target (15° saccades are shown in Fig. 2B and 20° in Fig. 2C). The monkey was rewarded for moving the eyes to within ±6° window of the visual target. After the injections this window was relaxed to ±7° and in some cases ±12° so that all attempted saccades to the visual target would be collected. The trajectories in each direction of Fig. 2C show five repetitions. Note the regularity, accuracy, and straightness of the trajectories. Following a control injection of saline in another monkey, the trajectories of the saccades were unchanged from baseline (compare Fig. 2D, saline, to Fig. 4A, same monkey 25° saccades, no injection). Filled circles show the average of all endpoints of control saccades to the same visual target. Saccades of five different amplitudes (5–25°; 8 randomized directions × 5 repetitions of each saccade + errors) were collected into separate files for each injection. Each file took ~6–15 min for the monkey to complete. A “complete” set of data covering all 40 positions was comprised of 5 files (1 for each amplitude, total collection time of 30–50 min). The amplitudes sampled during the first two or three files were repeated at the end of the sequence to document changes that occurred while the drug had diffused.

**Data analysis**

Following each experiment raw eye movement records were processed by software that identified the beginning and end of each eye movement using a template matching algorithm (Waitzman et al. 1991). Each trial was visually inspected, and marks indicating the
beginning and end of horizontal and vertical components of each saccade were corrected as needed. Corrective saccades following the primary movement were specifically excluded from the current analysis. Variations in saccade amplitude and direction following the injections were evaluated in a number of ways. One analytic technique calculated the fractional change in saccade amplitude and direction following the injection (Fig. 4D). A “difference coefficient” for each of these metrics was calculated by taking the difference between post- and pre-parameters and then expressing this as a fraction of the prevalue. This technique effectively normalized the data so that changes in eye movements of different amplitudes or directions could be compared. A negative value for the difference coefficient for amplitude (Diff. Amp.) indicated saccade hypometria, and a positive value reflected saccade hypermetria. Difference coefficients were plotted against target direction. In the analysis of changes following an injection, we also tested the “null” hypothesis that saccades in a particular direction were not deviated from their normal trajectory (Fig. 4E). If direction was not modified, then the direction at saccade end should be no different from target direction. The absolute difference between the angle at saccade end and target direction was the saccade deviation that was plotted as a function of target direction. If the deviation was positive (i.e., the postinjection angle was larger than target direction), then by definition this was plotted as a counterclockwise deviation (CCW), and if the deviation was negative (i.e., postinjection angle smaller than target direction), then this was scored as a clockwise (CW) deviation.

Two midbrain structures could be influenced by inactivation of the MRF by muscimol: 1) the nuclei of the optic tract (NOT) and 2) the INC. Contraversive slow phases of nystagmus develop after inactivation of the NOT, and position-dependent vertical post-saccadic drift occurs after inactivation of the INC (Cohen et al. 1992; Crawford and Vilis 1993; Crawford et al. 1991). To calculate the slow-phase eye velocity, instantaneous eye velocity was averaged from the end of the current saccade to just before the beginning of the subsequent saccade. This was done for the horizontal component of all spontaneous saccades (in total darkness) that occurred just before the paradigm began (including control injections of saline). The horizontal slow-phase eye velocity plotted for a single time point represented the average of all intersaccadic intervals for a particular file (~70–100 movements per file spanning 5–10 min). Time points were collected starting just before the injection and for each subsequent file following each injection until recording ended.

Drift amplitude [the amplitude of slow movement from saccade offset to the end of the drift as per Crawford and Vilis (1993)] was measured for at least 10 spontaneous eye movements occurring in each file. A running average (Student’s t-test) was used to decide when significant vertical drift had occurred.

Direction is directly proportional to vectorial amplitude for pure horizontal saccades (Fuchs 1967). However, for oblique saccades component stretching occurs to produce saccade trajectories that are straight. As a result, component (horizontal or vertical) duration is proportional to vectorial amplitude (King et al. 1986) and is used to display the duration data here. Comparison of the slopes of saccade duration versus vectorial amplitude was made by t-test to determine whether a change in component duration had occurred after muscimol injections. In a similar fashion, the log relationship between vectorial amplitude and velocity (Fuchs 1967; King et al. 1986) was compared before and after muscimol to decide whether saccades had been displaced off this main sequence.

Histology

Once all data were collected and the most productive eye movements regions identified, a pressure injection of 1–2 μl of fluorescent labeled microspheres (green and red, LumaFluor, ~0.05 μm diam; blue, Polyscience, BB19773, 0.05 μm diam) was made to positively localize the sites of microinjection in three monkeys. The location of the electrode tracks in one monkey was identified by placement of a small electrolytic lesion. At the conclusion of the experiments, monkeys were deeply anesthetized with pentobarbital sodium and perfused. The brains were removed, and 50 μm vibratome sections were made through the brain stem. Unstained sections (with fluorescent beads) were mounted wet and photographed under both white and fluorescent light. Alternating sections were stained with thionin and drawn onto paper using an inverted microscope. Drawings were then scanned into the computer and traced to produce the final anatomic representation of injection sites.

RESULTS

Neuronal effects of muscimol: areas of inactivation

Eight injections of the GABA A agonist muscimol were made in four monkeys at sites in the MRF where eye movement–related cells were recorded (Table 1). Of the eight injections, seven were made in head-fixed animals, and these injections were used to summarize the effects of muscimol inactivation. The eighth injection was done in the head-free animal to demonstrate the interaction between head and eye initial position shift. Besides these eight injections, two injections of inactive muscimol (determined empirically) produced no changes in eye movements and were used as controls. Changes in eye movements were noted as early as 5 min after a 1.0 μg injection of muscimol (Sigma, 0.5 μg/μl in sterile NaCl) into the MRF and could last for up to 7 h. Typically, electrical silence was noted at 20–30 min, and thus early time points were repeated after this initial inactivation period. Data collect-

### Table 1. Caudal cMRF muscimol injections

<table>
<thead>
<tr>
<th>Injection</th>
<th>Amount, μl</th>
<th>Concentration, μg/μl</th>
<th>Side</th>
<th>Head Tilt</th>
<th>Nystagmus Onset</th>
<th>Onset of Effects</th>
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<tbody>
<tr>
<td>g021794</td>
<td>1</td>
<td>0.5</td>
<td>Left</td>
<td>Yes/Right</td>
<td>P &gt; 0.05</td>
<td>5 min; hypermetric; up and right</td>
</tr>
<tr>
<td>k032995</td>
<td>1</td>
<td>0.5</td>
<td>Left</td>
<td>Yes/Right</td>
<td>P &gt; 0.05</td>
<td>No effects</td>
</tr>
<tr>
<td>k033195</td>
<td>1</td>
<td>0.5</td>
<td>Right</td>
<td>No</td>
<td>P &gt; 0.05</td>
<td>50 min; spontaneous; up and left</td>
</tr>
<tr>
<td>k040395</td>
<td>1</td>
<td>0.5</td>
<td>Left</td>
<td>No</td>
<td>P &gt; 0.05</td>
<td>30 min; spontaneous; up and right</td>
</tr>
<tr>
<td>c041696</td>
<td>0.5</td>
<td>1</td>
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<td>Yes/Right</td>
<td>P &gt; 0.05</td>
<td>44 min; hypermetric; up and right</td>
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<td>1</td>
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<td>Yes/Left</td>
<td>33 min, P &lt; 0.05</td>
<td>59 min; hypermetric; up and left</td>
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<tr>
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<td>1</td>
<td>Right</td>
<td>Yes/Left</td>
<td>127 min, P &lt; 0.05</td>
<td>93 min; hypermetric; up and left</td>
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<td>t091798</td>
<td>0.675</td>
<td>1</td>
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<td></td>
<td>21 min; head and eye shift</td>
</tr>
</tbody>
</table>

Eight muscimol injections made in 4 monkeys. Rows without labels indicate a repeated dose of the given amount at the same injection site.
tion began with the start of the injection and continued for as long as the monkey could perform the behavioral tasks.

We made parallel tracks 1 and 2 mm away from a 1.0 μg/μl muscimol injection in one monkey. Data from these tracks showed that the blocked region (electrical silence) extended no greater than 1.5 mm laterally from the site of injection. Cell activity 2 mm lateral to the injection was normal. Monitoring of activity above and below this site demonstrated a vertically blocked region of 2.5 mm above the site of the injection. No eye movements could be elicited from within the blocked region using electrical microstimulation at 3 times threshold, but eye movements could be elicited below the blocked region. Twenty-four hours later, neuronal activity in the blocked area had recovered, electrical microstimulation could elicit saccades, and eye movements had returned to normal. These experiments suggested that an injection of 1.0 μg/μl of muscimol inactivated an ellipsoid portion of the brain stem 2.2 mm in diameter and 1.5–2.5 mm in length. After a control injection of saline, neuronal activity was suppressed for ~3 min (Fig. 2D, monkey G), but returned to normal levels within 5–10 min. Following this control injection, saccades to the eight different target positions located 20° from primary position were straight, accurate, and thus unaffected by the injection (Fig. 2D).

Our initial hypothesis was that the MRF [corresponding to nucleus cuneiformis and nucleus subcuneiformis of Olszewski and Baxter (1954)] was physiologically a homogeneous region. As our experiments progressed, it was clear that some division of the “MRF” was necessary, because the effects on eye movements were quite different if injections were made rostral or caudal to the posterior commissure. Specifically, an analysis of caudal injection sites showed that oblique, upward, contraversive saccades became hypermetric (Fig. 3, all caudal injections), whereas vertical saccades became hypometric after rostral injections (Fig. 4A of accompanying paper, Waitzman et al. 2000).

**SUMMARY OF CAUDAL INJECTIONS**

1. g0217
2. k0329
3. k0331
4. k0403
5. c0416
6. c0419
7. c0521
8. t0917

**FIG. 3.** One representative section of the caudal brain stem showing the locations of all 8 muscimol injections made in 4 monkeys (•). All of these injections produced saccade hypermetria, and their results are presented in the current paper. Abbreviations for all anatomic sections: III, 3rd nerve nucleus; IV, trochlear nucleus; aq, aqueduct of sylvius; BC, brachium conjunctivum; BCdec, decussation of the brachium conjunctivum; BIC, brachium of the inferior colliculus; BSC, brachium of the superior colliculus; CG, central gray; HBL, medial habenular nucleus; inc, interstitial nucleus of Cajal; LL, lateral lemniscus; ML, medial lemniscus; MLF, medial longitudinal fasciculus; NOT, N. of the optic tract; NPC, N. of posterior commissure; PB, N. parabigeminus; PC, posterior commissure; riMLF, rostral interstitial nucleus of the MLF; RPC or NRPC, N. reticularis pontis caudalis; RTP or NRTP, N. reticularis tegmenti pontis.
Inactivation of the ventral-caudal MRF (the cMRF): synopsis

Muscimol inactivation of the ventral-caudal MRF produced seven primary oculomotor effects in the monkey: 1) hypermetria of contraversive oblique saccades; 2) reduced saccade latency; 3) a moderate reduction in saccade duration, with an increase in saccade velocity following many injections; 4) repetitive macrosaccadic square-wave jerks to a specific goal in the orbit; 5) spontaneous saccades in the dark directed toward the same specific goal in space (relative to the head); 6) straight trajectories of saccades directed toward the orbital goal and curved trajectories of saccades directed toward adjacent locations to the goal; and 7) contraversive head-tilt following five of eight ventral-caudal injections.

These effects were consistent across monkeys and did not occur after inactivation of adjacent locations in the brain stem. The effects on saccade metrics (duration, velocity, and latency) will be illustrated for seven injections. The eighth injection was made in a monkey free to move its head, and thus the data for cMRF metrics were not comparable to the head-fixed case. Careful examination of saccade duration will point to which portions of the oculomotor models could account for the observed changes. No change in saccade duration would suggest the input to the local feedback loop had shifted, whereas increased duration would suggest loss of feedback. Shorter duration and higher saccade velocity suggest a combination of effects on model parameters.

Analysis of square-wave jerks and the goal-directed nature of postinjection saccades are presented for all injections made in head-fixed animals. Each of these injections had a different goal to which spontaneous saccades were directed repeatedly. Data from two injections will be presented in detail, one in the left and the other in the right MRF. The rest of the data are presented in summary format to illustrate the range of effects observed.

Inactivation of the cMRF: changes in saccade metrics

Seven injections (c0416, c0419, c0521, g0217, k0329, k0331, and k0403) placed into the caudal MRF of three head-fixed monkeys produced hypermetric saccades. The results of one muscimol injection (1 µg/2 µl) placed at the site of cMRF long-lead burst neurons that discharged before contraversive (rightward) saccades is shown in Fig. 4. Multiunit contraversive eye movement related activity was registered through the recording syringe at a similar depth at which the single cells of Fig. 4F (movement fields) had previously been recorded. Electrical microstimulation was not performed at this site. Within 5 min after the end of the injection (duration of 20 min), 25° saccades up and to the contraversive side became hypermetric (Fig. 4B, positions 1 and 2; ○, averaged endpoints of control saccades). During the next hour of observation (5–30 min shown) all visually guided saccades up and to the right became hypermetric (Fig. 4, B–D). This hypermetria affected the vertical more than the horizontal component of movement (Fig. 4D). For a 15° oblique saccade the horizontal component of movement was increased by 20%, whereas the increment in the vertical component approached 50% (Fig. 4D, compare ○ with □).

There was a counterclockwise, upward deviation of the endpoints of contraversive, horizontal (position 0), and oblique, upward saccades (position 1, 45°) after this injection. The endpoints of pure upward movements (position 2, 90°) were deviated downward (i.e., negative direction, clockwise in Fig. 4E). A similar, albeit smaller reversal of saccade endpoint deviation occurred between positions 5 (225°) and 6 (270°; Fig. 4E). These reversals of saccade deviation correspond with zero crossings from counterclockwise to clockwise (Fig. 4E, arrows). This defined a plane tilted ~25° from the vertical toward which saccade endpoints were deviated (Fig. 4B). This plane also influenced the trajectory of the saccade. Saccade trajectories close to the plane remained almost straight, whereas saccade trajectories in other directions became curved. For example, the trajectories of upward vertical saccades to position 2 were bowed away from this plane (but their endpoints were closer to the plane), whereas the trajectories of oblique saccades to position 1 and those of horizontal saccades were bowed upward toward this plane. Similarly, downward saccades to position 6 were bowed away from the tilted vertical plane (Fig. 4B). Such curvature suggests discoordination in the generation of the horizontal or vertical components of the saccade such that the vertical component reached peak velocity before the horizontal component.

Details of the upward saccade hypermetria following this injection (g0217) are shown in Fig. 5. Hypermetric oblique saccades (position 1) had an increase in peak velocity compared with preinjection movements (Fig. 5A, horizontal; Fig. 5B, vertical). Saccades in the opposite direction (down and to the left, position 5) were only slightly hypometric (Fig. 5D). In both cases (position 1 and 5), the amplitude and velocity of the vertical component was affected more than the horizontal component. In fact, horizontal component amplitude for ipsiversive saccades (position 5) was slightly hypometric (Fig. 5C, solid line). The overall increases in vectorial peak velocity for both directions (positions 1 and 5) were matched by a commensurate increase in saccade amplitude and duration. As a result, these postinjection saccades remained on the amplitude versus peak velocity main sequence (Fig. 5E, Table 2, P > 0.05).

The increased amplitude of postinjection saccades was matched by a commensurate increase in horizontal saccade duration. This maintained the same linear amplitude-duration relationship as before injection [Fig. 6A, slopes (m) not different]. However, duration of the vertical saccade component was longer than the associated vectorial amplitude would have required (Fig. 6B), while the slope of postinjection vertical duration versus amplitude relationship rose. The difference in slope did not reach statistical significance (see Fig. 10C). On the other hand, the latency to onset for saccades of all amplitudes was significantly reduced following this injection. Contraversive, upward saccades were initiated the fastest and some latencies (150 ms) approached that of express saccades (Fig. 6C).

To determine whether muscimol had spread to include the NOT, dorsal to the MRF, horizontal slow-phase eye velocity (slow movements between saccades) was calculated after the injection (see METHODS) (Cohen et al. 1992). Control preinjection files demonstrated <3°/s of contraversive, slow-phase eye velocity (Fig. 6D, ○). Following this muscimol injection, no contraversive horizontal nystagmus was found (Fig. 6D, ●). These results suggested that the changes in saccade amplitude, velocity, and latency could not be accounted for by spread of the muscimol to involve the nucleus of the optic tract.
FIG. 4. Hypermetric saccades developed after a muscimol injection in the caudal MRF of monkey G (g0217). A: control trajectories before injection. B: VGS trajectories 5 min following the injection of 1 μg (2 μl of 0.5 μg/μl) of muscimol into the left MRF. Note contraversive (rightward) saccade hypermetria to target positions 1 and 2. C: large almost 25° hypermetric saccades generated in direction 1 to a visual target that was located 10° up and to the right. Times indicate the onset of eye movement recording after muscimol injection. F, averages of all preinjection control eye positions endpoints. D: vectorial difference coefficients for 4 different amplitude VGS in each of 8 target directions. Horizontal and vertical difference coefficients for 15° VGS in each of 8 directions. Different symbols indicate which amplitude was affected by the injection. E: deviation of the endpoints of saccades occurred after the muscimol injection. Key in D applies to E. CW, clockwise rotation of saccade endpoints are shown along the ordinate (negative); CCW, counterclockwise rotation of saccade endpoints are shown along the ordinate (positive). F: 2-dimensional cartesian plots of the contraversive (right) movement fields of 3 neurons recorded from the left MRF at the site of this muscimol injection. G: site of this injection (g0217) and a control saline injection (g0223). Abbreviations as in Fig. 3.
Inactivation of the cMRF: square-wave jerks and changes in initial position

At the end of 25 min after the injection, the monkey developed pronounced contraversive, upward macrosaccadic square-wave jerks (Fig. 7). The requirement of this particular paradigm was for the monkey to maintain stable fixation (Fig. 7, control, dotted line: see also Fig. 2A). After the injection the monkey made repeated saccades that were in the same direction as the previously described hypermetria. Each eye movement was separated by a minimal intersaccadic time interval of 150–200 ms. These movements were very stereotypic and brought the eye to a specific location in the orbit (Fig. 8A).

Although changes of visually guided saccades with shifts in initial position were not specifically studied in this monkey, spontaneous saccades made in total darkness were collected just after this fixation file. The trajectories of the spontaneous saccades (whose vectors are shown in Fig. 8B) demonstrate that the eyes were directed to a specific goal in the orbit located 13° to the right and 19° up (error bars are ±1 SD). We compared an average of the endpoints of the macrosaccadic square-wave jerks from the fixation paradigm with the location...
of the endpoints of all of the spontaneous saccades and found an extensive overlap (compare rectangular boxes in Fig. 8, A and B). The dependence of postinjection spontaneous saccades on initial eye position was assessed by calculation of an "orbital perturbation index." This reflects the slope of the regression line relating component saccade amplitude with initial position (Russo and Bruce 1993). The indexes for horizontal and vertical saccade components were markedly elevated, supporting a strong effect of initial eye position on saccade amplitude. In summary, this injection demonstrated that inactivating the cMRF was critical for the generation of saccade hypermetria. Within 1 h of this injection the monkey generated repetitive macrosaccadic square-wave jerks that brought the eyes to a specific goal in the orbit. Two hours after the injection, the monkey’s head was released and a contraversive head tilt was noted.

Table of log regression coefficients for the amplitude-velocity (main sequence) relationship following each injection. $r^2$ (log) is the correlation coefficient for the log model.

**Table 2. Main sequence for caudal injections**

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<th>P Value</th>
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<tr>
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Summary of results: caudal injections

All but one muscimol injection showed a significant reduction in saccade latency (Fig. 10). The higher control latency for monkey C was most likely the result of difficulty with down gaze in the right eye (the recorded eye) following surgical procedures to correct tethering of the eye-coil in this eye. However, the reduction in latency was significant, and control latency returned back to 600 ms the day following the c0416, c0419, and c0521 injections. Taken together, these latency results suggest a role for the ventral-caudal MRF in saccade initiation and maintenance of fixation.

Because of the differential effect on saccade velocity following the two injections shown in detail, we also examined the duration of the saccades for each of the seven injections. Note that in six of seven injections, horizontal saccade duration was reduced and in five of the seven injections, vertical saccade duration was reduced compared with control. These trends reached significance in only two of the horizontal and one of the vertical measurements (Fig. 10, B and C). This corresponded closely to an increase in saccade velocity that was higher than expected for amplitude and positioned these movements above the main sequence. These results suggest that inactivation of the ventral-caudal MRF could influence portions of the saccade burst generator.

Effects of muscimol inactivation of the caudal MRF on the amplitude of postinjection saccades are shown in Fig. 11. To compare the degree of hypermetria of one injection to that of another, the difference coefficient data were plotted so that the direction of hypermetria was up and to the right (i.e., 45°). Thus all injections were displayed as if they had occurred on the left side of the brain stem. Difference coefficients for the time point for which the monkey demonstrated the greatest hypermetria but was still capable of making saccades in all other directions are illustrated. All MRF injections caudal to the posterior commissure produced contraversive saccade hypermetria, albeit to a small degree in two injections (k0329 and k0403). The direction of saccade hypermetria was primarily upper and to the contraversive side. In one case, horizontal saccades were hypermetric (c0419, ○), but most often oblique upward saccades were hypermetric. This family of curves illustrates two points. First, the saccade hypermetria following ventral-caudal MRF inactivation ranged from ~5% of the control value to >50%. Second, there was a small secondary peak in saccade hypermetria 180° in the opposite direction of the primary hypermetria. This occurred for all injections except k0403, which had a small degree of hypometria in the opposite direction.

A final aspect of the hypermetria was that it directed saccades toward a specific region in the orbit regardless of initial eye position. This was demonstrated by analyzing the direction and final endpoints of files of spontaneous saccades recorded...
toward the end of the monkey’s ability to generate visually guided saccades. The different regions to which spontaneous saccades were directed are summarized in Fig. 12 for seven injections and two control days. Regions determined by right-sided MRF injections are shown by open symbols, and those following left-sided injections are filled. Note that all regions were located in the top half plane of movement and most (6 of 8) were contraversive. This trend of spontaneous saccades toward a specific goal in the orbit was confirmed by calculating an orbital perturbation index for both the horizontal and vertical components of spontaneous saccades. A clear effect \(P < 0.05\) of initial eye position on the vertical component of spontaneous saccades was found in three of seven injections (data not shown). The horizontal orbital perturbation indexes were increased over control (5 of 7) but did not reach statistical significance.

**Head tilt and shift of initial eye position**

Three of eight injections in the ventral-caudal MRF were associated with a shift in initial eye position (Fig. 13). The
shift in initial eye position increased over time, and typically the monkey made no attempt to compensate for the shift. The shift was contraversive in two injections (c0419 and c0521) and ipsiversive and up after one injection (c0416).

One key to a better understanding of the changes noted in initial eye position came from studying the contralateral head tilt generated after the ventral-caudal muscimol injections. One possibility was that the shifts in initial position noted with the head fixed were compensatory for an attempted head movement in the opposite direction. Another possibility was that the MRF contributed to maintenance of initial eye position via its connections to the omnipause region of the PPRF. Loss of a fixation signal would produce destabilization of fixation similar to the macrosaccadic square-wave jerks shown above (Fig. 7). To examine whether the shift in initial eye position was compensatory, we measured the head tilt in one monkey free to move its head following a muscimol injection in the right ventral-caudal MRF.

With the use of an additional coil fixed to the head in the coronal plane, horizontal and vertical, but not torsional displacement of the head could be recorded (only 2 coils, 1 eye, and 1 head could be monitored). An almost immediate contralateral head roll of ~30° was confirmed visually and via photographs. The coronal coil demonstrated a contraversive and downward head displacement (Fig. 14, D, E, and G). This was associated with a compensatory shift of the initial position of the eyes up and to the ipsilateral (right) side (Fig. 14D, ●; F, horizontal; ■; H, vertical). This combination of head tilt and shift in eye position resulted in gaze (combination of head and eyes) being directed toward the center of the screen (Fig. 14B). This injection was performed using <0.5 μl of muscimol from a picospritzer apparatus, limiting spread of muscimol to adjacent structures. This suggests that the shift of initial eye position seen after the muscimol injections could have been compensatory for an intended head tilt.

**DISCUSSION**

To better characterize the oculomotor function of neurons in the MRF, injections of the GABA A agonist, muscimol, were placed at the sites of midbrain neurons that discharged with, or where electrical microstimulation induced, contraversive, conjugate saccades (Cohen et al. 1985; Handel and Glimcher 1997; Waitzman et al. 1996). Two previous, careful studies of single-neuron activity in behaving monkeys have revealed only a gross topographic arrangement of the oculomotor functions in this region. In particular, cMRF neurons, adjacent to the oculomotor nuclei, began to discharge 150 ms and peaked 8–10 ms before saccades with a contraversive horizontal or downward oblique component of movement (Waitzman et al. 1996). More rostrally located MRF neurons, adjacent to the oculomotor nuclei, began to discharge 150 ms and peaked 8–10 ms before saccades with a contraversive horizontal or downward oblique component of movement (Waitzman et al. 1996). More rostrally located MRF neurons, adjacent to the INC also had long-lead activity, but were most sensitive to contraversive oblique and vertical saccades (Handel and Glimcher 1997). The movement fields for both groups of neurons were large and could extend for up to 40°.

The primary findings of this study were that inactivation of the ventral-caudal MRF 1) generated conjugate, contraversive, upward saccade hypermetria; 2) reduced saccade latency; and 3) produced a moderate increase in saccade velocity accompanied by a moderate reduction in saccade duration. Many ventral-caudal injections also produced macrosaccadic square-wave jerks that repetitively brought the eyes to a fixed place in the orbit. Similarly, spontaneous saccades executed in total darkness were directed toward the same specific orbital position. The distribution of the orbital goals was across the contralateral upper field of movement. Interestingly, three of the muscimol injections induced a displacement of the initial position of the eyes. These findings suggested a number of possible roles for cells in the cMRF: 1) participation in feedback of an eye position or displacement signal, 2) stabilization/maintenance of fixation, and 3) activation of the saccade burst generator. These results are discussed in light of the various anatomic connections of the MRF and how these cells could...
participate in the circuitry needed for the control of saccades and stabilization of fixation. Simulations of the two models presented in the introduction are used to demonstrate that an eye position and not eye displacement model can best explain the current observations.

Localization of muscimol inactivation

GABA containing interneurons have been localized to the MRF (Nagai et al. 1983). Our basic assumption was that muscimol activation of GABAergic synapses on saccade related long-lead burst neurons in the MRF produced the observed oculomotor effects and left fibers in passage (i.e., the central tegmental tract) unaffected (Andrews and Johnston 1979; Krogsgaard-Larsen et al. 1979; Ritchie 1979). Single-unit recordings through and around the region blocked by muscimol demonstrated no neural activity in a sphere of radius 1.1 mm centered on the site of injection and support this view. Saline injections produced no oculomotor effects, thus eliminating a mechanical pressure gradient as the source of our findings (Fig. 2). Inadvertent inactivation of a number of oculomotor structures adjacent to the MRF including the nucleus reticularis tegmenti pontis (NRTP), the SC, and the NOT, could color the above interpretation of our results. However, muscimol inactivation of these regions has produced little or no saccade hypermetria (Aizawa and Wurtz 1998; Hikosaka and Wurtz 1985a; Lee et al. 1988; Munoz and Wurtz 1993; Quaia et al. 1998; van Opstal et al. 1996). Destabilization of fixation has occurred following rostral SC inactivation, but macrosaccadic square-wave jerks directed toward a specific location in the orbit were not generated (Munoz and Wurtz 1993). Possible inactivation of the NOT could produce horizontal, contraversive nystagmus (Cohen et al. 1992). In five of seven injections, no nystagmus was found (Table 1). In one of the other two remaining injections, onset of hypermetria occurred before contraversive nystagmus developed. Taken together this con-
stellation of findings suggests that inactivation of the MRF and not adjacent structures produced saccade hypermetria.

Oculomotor functions of cMRF: anatomic connections

Intracellular filling of MRF neurons has shown them to be of at least three types (Scudder et al. 1996). RTLLBNs located within the central MRF (i.e., the nucleus subnucleiformis) direct their axons toward the ipsilateral SC where they arborize within the intermediate and deep layers. These cells provide a collateral branch that crosses the intracollicular commissure to innervate the contralateral SC (Moschovakis et al. 1988). This group of neurons could influence the generation of saccades in the SC. A second group of MRF LLBNs [probably reticulospinal LLBNs (RSLLBNs)] is located lateral to the INC, rostral to the RTLLBNs just described (Scudder et al. 1996). The RTLLBNs have contralateral movement fields, whereas the RSLLBNs have vertical movement fields (Handel and Glimcher 1997; Scudder et al. 1996; Waitzman et al. 2000). These cells have axons that descend toward the pons to innervate the raphe nuclei (raphe pontis, nucleus RIP, raphe obscurus) and the medullary reticular formation (primarily the inhibitory burst neuron region caudal to the abducens nucleus) (Scudder et al. 1996). The RSLLBNs could interact with both saccade and head generation networks in the pons and spinal cord (see accompanying paper, Waitzman et al. 2000). Scudder and colleagues (1996) have also described a third group of saccade-related neurons whose cell bodies are probably located within

FIG. 9. Generation of repetitive macrosaccadic square-wave jerks following a 2nd muscimol injection (1.0 µl of 1.0 µg/µl) in the right caudal MRF (c0521). A: control saccade trajectories. B: trajectories of saccades made to eight 25° targets 88 min after a muscimol injection in the right, caudal MRF. Note moderate saccade deviation and hypermetria of saccades to position 3. C, average location of control saccades. D: a similar file of 5° visually guided saccades collected 127 min after the muscimol injection. Note the marked hypermetria of contraversive saccades made to a 5° target at position 3. D: macrosaccadic square-wave jerks that interrupted steady fixation at a target located at the center of the tangent screen. The center of the rectangular box corresponds to the average, and its edges are ±1 SD of final eye position for the entire group of square-wave jerks. E: spontaneous saccades made in total darkness 142 min after this muscimol injection. Note that the saccades were directed toward a goal whose center was located 11.8° to the left and 18.4° up. The rectangular box around these endpoints is determined as in D. Note extensive overlap with endpoints of saccades in D.
A: Average Vectorial Latency for Caudal Muscimol Injections

FIG. 10. Summary of changes in saccade latency and duration following muscimol inactivation of the caudal MRF. Data from 7 muscimol injections are included of which 4 were performed on the left side and 3 were placed in the right MRF (c0519, c0521, k0331). A: note the reduction in latency of all saccades with the exception of one experiment (k0331). The high preinjection latencies for monkey C were noted after an eye coil repair that slowed downward saccades. The postinjection reduction in saccade latency for the 3 injections in this monkey was still significant when downward saccades were excluded from the analysis.

B: slopes of the component duration vs. vectorial amplitude graphs as shown in Fig. 6B (horizontal, B, and vertical, C) before and after injection. Horizontal saccade duration was reduced for all injections with exception of k0403, but reached significance for c0416 and c0419. C: a similar reduction in vertical component duration for all injections with the exception of c0521 and g0217, both of which showed moderate increases. Only the reduced duration of postinjection saccades of c0416 reached statistical significance.
the caudal MRF (i.e., again nucleus subcuneiformis) and whose discharge is similar to the RTLLBNs. However, the axons of these cells (cRSLLBNs) were directed caudally within the predorsal bundle and innervated the NRTP, RIP, the nucleus reticularis pontis oralis and caudalis (NRPo-NRPc; including the excitatory and inhibitory burst neurons) and sent a descending axon to cervical levels of the spinal cord. An ipsilateral projection from the cMRF and cuneate reticular nucleus to the ventral horn of the cervical spinal cord, separate from that of the INC, has been confirmed repeatedly in both cat (Castiglioni et al. 1978) and monkey (Crawford and Villis 1993; Fukushima 1987; Fukushima et al. 1987; Kokkoroyannis et al. 1996; Robinson et al. 1994; Scudder et al. 1996). Moreover, the descending MRF projections were more numerous than the projections from the INC to the cervical spinal cord (Robinson et al. 1994; Scudder et al. 1996). Furthermore, the MRF also receives head-related proprioception via direct afferents from the cervical spinal cord and dorsal column nuclei (Bjorkland and Boivie 1984; Pechura and Liu 1986). In sum, this pattern of connectivity suggests that caudal MRF neurons could participate in the generation of combined head and eye movements. The output of cRSLLBNs to the omnipause neurons in the RIP and the precerebellar saccade generating machinery in the NRTP and NRPo-NRPc could account for the marked reduction in saccade latency found after cMRF inactivation (J. Buttner, personal communication; Buttner-Ennever and Buttner 1988; Horn et al. 1994; Scudder et al. 1996). In the normal state, MRF activity could enhance the tonic firing rate of omnipause neurons thereby suppressing unwanted saccades as has been suggested by the results of electrical microstimulation.
lotion (Cohen et al. 1985). A likely candidate for this function would be high background cMRF neurons described previously (Waitzman et al. 1996). Loss of this excitation would lead to fixation instability. However, loss of fixation would not necessarily generate macrosaccadic square-wave jerks per se. More likely, the muscimol inactivation produced two possible effects. First, it could produce a loss of a suppression signal (i.e., reduced excitation) to the omnipause neurons, permitting more frequent and longer pauses. Second, during saccades, the MRF could provide a feedback signal (i.e., of current eye displacement or eye position) needed to stop the saccade accurately. A reduction or complete loss of this feedback signal would cause a persistent motor error that the saccadic system would try to correct, thus generating the repeated macrosaccadic jerks at short latency.

The interesting part of the square-wave jerks observed here were that they brought the eyes to a particular position in the orbit that was similar to the final positions of many of the spontaneous saccades made in total darkness. This could not be accomplished by feedback of an eye displacement signal.

**Caudal MRF: craniotopic not retinotopic organization**

Five findings in the current study suggest that MRF neurons are organized in a spatial not a retinotopic frame of reference. First, electrical stimulation at sites where muscimol was injected generated contraversive saccades. Recent results have demonstrated that electrical stimulation and single-unit recording at similar sites elicited goal-directed saccades (Waitzman et al. 1998). Muscimol inactivation of this region left horizontal saccades for the most part intact. The endpoints of horizontal saccades were deviated upward and in a few instances, the horizontal component of movement was modestly hypermetric. At the same time, contraversive upward, oblique saccades were markedly hypermetric. If the ventral-caudal MRF were retinotopically coded, then horizontal saccades should have been rendered hypometric and the vertical component of saccades should have been unaffected. This would be similar to the results of muscimol inactivation of the retinotopic map found in the superior colliculus (Aizawa and Wurtz 1998; Hikosaka and Wurtz 1985a; Lee et al. 1988; Munoz and Wurtz 1995a,b; Quaia et al. 1998).

A second finding following MRF inactivation was that spontaneous saccades were not executed to random locations in the orbit. Instead, saccade endpoints defined a specific “goal” in the orbit. This goal was typically displaced upward from the pure horizontal locus defined by prior electrical stimulation and single-unit recording (see Fig. 15). Third, an analysis of the endpoints of macrosaccadic square-wave jerks during fixation showed that the eyes were directed to the same locus defined by spontaneous saccades executed in total darkness. The amplitude and direction of these repetitive movements varied in a predictable way with shifts in the initial position of the eyes, such that their endpoints coincided with the same region of the orbit delineated by the final positions of spontaneous saccades.

Fourth, a moderate shift in the initial position of saccades was noted after three of eight injections. Last, a contraversive head tilt was observed after nearly all the caudal MRF injections. Electrical stimulation in the MRF of dogs and monkeys has produced contraversive saccades in association with head movements (Bender and Shanzer 1964; Silakov et al. 1999; Szentagothai 1943). Electrolytic lesions in the MRF produced an ipsilateral gaze preference, in which monkeys did not make gaze movements (head and eyes) to visual stimuli on the contralateral side (Komatsuzaki et al. 1972). Taken together, these data are most consistent with the idea that MRF neurons participate in the generation of a final eye or target position in the orbit (craniotopic, Eye Position Model), rather than an eye displacement signal (retinotopic, Eye Displacement Model).

The concept that the MRF specifies final orbital position would fit with a number of other physiological and anatomic findings. The cMRF receives projections from both cortical and subcortical regions where goal-directed saccades have
FIG. 14. Head tilt and initial position changes 21 min after a muscimol injection in the right MRF of a head-free monkey (t917). A and C: preinjection initial gaze, eye, and head positions. Gaze is centered. Head and eye positions are distributed around initial eye position. D: 30 min after muscimol injection there was a downward and small contraversive shift in initial head position (open circles) measured by a head coil in the coronal plane. Initial eye position (gray circles) was shifted upward and to the ipsiversive side. Gaze (filled circles, B) showed no change. E–H: histograms of initial head (E, horizontal, mean \(1.86 \pm 3.56\); G, vertical, mean \(4.50 \pm 4.47\)) and eye position (F, horizontal, mean \(-2.46 \pm 2.68\); H, vertical, mean \(-2.82 \pm 4.31\)) before (open bars) and initial head (E, horizontal, mean \(20.47 \pm 3.04\); G, vertical, mean \(8.15 \pm 3.14\)) after (filled bars) injection. These histograms show the significant difference in initial head and eye positions that exactly compensated for each other. The postinjection means of both initial eye and head positions were significantly different from control at the \(P < 0.05\) level.
have open and closed movement fields similar to those found in the SEF (Schall 1991). Recent data suggest that the discharge of some SEF neurons is object oriented and initial eye position can have a moderate effect on discharge (Olson and Gettner 1995; Russo and Bruce 1993). Microstimulation of the SEF produces contraversive saccades that bring the eyes to a "termination zone" (Russo and Bruce 1993; Tehovnik et al. 1994). This is similar to the variable amplitude and/or goal-directed saccades generated after MRF microstimulation (Cohen et al. 1985; Silakov et al. 1995; Waitzman et al. 1998). In conclusion, we suggest that muscimol inactivation of the cMRF uncovered an underlying craniotopic organization of neurons that contributes to the generation of an estimate of a final eye or target position in the orbit signal.

**Generation of vertical saccade components from a “horizontal” region**

Single-unit recordings and microstimulation have strongly implied that neurons in the caudal MRF participate in the generation of the horizontal component of saccades (Cohen et al. 1985; Waitzman et al. 1996). Our expectation was that inactivation of this caudal region would have generated hypometric horizontal not the hypometric oblique saccades observed. This prediction was based on two primary assumptions. First, we hypothesized that the MRF was coded in retinotopic coordinates, and, second, we thought MRF neurons provided signals for generation of eye movements alone, not a combination of head and eye movements. The data just reviewed support the idea that MRF neurons are organized in craniotopic not retinotopic coordinates. We now present one example of how inactivation of a brain stem region that specifies final horizontal position of the eyes in the orbit could produce oblique saccades.

An averaged representation of the movement fields from nine caudal MRF neurons from one monkey was constructed by normalizing all neuronal activity to the peak firing of each cell (see also Fig. 9C of Waitzman et al. 1996). The peak activity of this averaged movement field was greatest for contraversive eye positions (Fig. 15, positive horizontal axis is contraversive). However, contraversive movements, with vertical components were also associated with increased (albeit lower than peak) neuronal discharge (note elevated shelf of neural activity on the right half of the movement field). Moreover, there was no clear cutoff of activity for saccades of very large amplitude (i.e., the movement field was open). Ipsiversive movements were associated with little or no activity. Unfortunately these MRF neurons were not studied during shifts of initial position, but for the purposes of this discussion we assume that changes in initial position would lead to activation of cells when the eye reached a particular final position. We also made the assumption that muscimol inactivation would eliminate neural activity in this averaged “movement field” of all saccades with final positions, which landed on the contraversive side 10° up to 40° down. The resultant averaged activity is shown in Fig. 15B. Note that a relative peak activity of the grouped MRF activity appears up and to the contraversive side. We further assume that muscimol inactivation of the caudal MRF uncovered an underlying craniotopic organization of neurons that contributes to the generation of an estimate of a final eye or target position in the orbit signal.

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**FIG. 15.** Changes of averaged cMRF neural activity before and after muscimol inactivation. A: averaged activity of 9 cMRF neurons recorded from *monkey G* before muscimol injection. Target position in the horizontal and vertical direction is shown on the x-y plane. Neuronal activation, normalized to individual peak activity and combined across all cells is shown along the z-axis. A high level of spontaneous background activity of MRF neurons is apparent from the shelf shown across the contraversive portion of the x-y plane. Note a large contraversive peak of activity for primarily horizontal saccades of 40–60° final position. B: large “relative” peak of activity appears after hypothetical suppression of MRF activity related to down and contraversive saccades (cutoffs were set at <10° up and 0° horizontal). This suggests that resultant saccades would be directed up and to the contraversive side (site of peak activity). cMRF neurons and their associated best saccade vectors used for the plot in A were g1203, 14.5°; g1207, 1.1°; g1209, –10.9°; g1210, 14.4°; g1221, –67.9°; g0309, –56°; g0310, –54°; g0310.2, –65.6°; g0328, –8.9° (negative angles relative to 0° right).

been elicited. Electrical microstimulation and single-neuron recording in the SC and the FEF, both of which provide projections to the cMRF, have demonstrated a moderate effect of initial eye position on the size of elicited saccades (Cowie and Robinson 1994; Segraves and Goldberg 1991). Probably more important are the large numbers of projections from the supplementary eye fields (SEF) (Huerta and Kaas 1990; Shook et al. 1990) and posterior parietal cortex (Kunzle and Akert 1977; Leichnetz and Goldberg 1988) to the MRF. Neurons in the SEF discharge in response to purposeful movements and have open and closed movement fields similar to those found...
position of spontaneous and visually guided eye movements observed after the injection of muscimol.

Evidence of a contraversive head tilt following MRF inactivation suggests that interaction of the MRF with either the vestibular or head movement control system could produce vertical saccade components. Interactions with the vestibular system would have to occur via indirect pathways because there are no direct projections from the MRF to the vestibular nuclei or vestibular cerebellum (flocculus). On the other hand, projections from the MRF to regions of the brain stem and spinal cord that participate in head movements have been described. Two regions could have particular importance. First, the loss of MRF input to the cervical spinal cord (Castiglioni et al. 1978; Robinson et al. 1994) could produce an intended contraversive head tilt (i.e., the monkeys were head fixed). This could generate secondary changes of proprioceptive inputs to the vestibular cerebellum and would result in a reduction of floccular output. This scenario would result in the generation of a compensatory upward vertical output signal. A more direct route might target the NRTP, which also receives significant projections from the MRF as well as the SC and cerebellar nuclei (Brodal 1980; Gerrits and Voogd 1986; Hartsig 1978; Scudder et al. 1996; Thielert and Their 1993). Inactivation of the caudal portion of NRTP would produce a loss of torsional eye control and could result in the generation of an upward eye movement component (van Opstal et al. 1996). A similar mechanism has been proposed for the upward deviation of saccade trajectories observed after inactivation of the SC (Aizawa and Wurtz 1998; Quaia et al. 1998, 1999). In sum, generation of the vertical saccade component after muscimol inactivation would be better understood in head-free monkeys whose head and eye movements were recorded in three dimensions.

**Generation of saccade hypermetria: models**

What physiological mechanisms are responsible for saccade hypermetria, increased saccade velocity, and goal-directed macrosaccadic square-wave jerks observed after muscimol inactivation of the MRF? Simulation has demonstrated that changes at three sites (Hyper #1, #2, and #3) in the Eye Position model (Fig. 1A) and two sites in the Eye Displacement model (Fig. 1B) could generate saccade hypermetria. Manipulation of two locations (Hyper #1 and #2), the model input and the local feedback pathway, could generate saccade hypermetria in both models (Hyper #1 and #2). A shift in the input to either model (i.e., a new eye displacement, or desired final eye position) to a higher value resulted in saccade hypermetria (Fig. 16A, new input).

In the one-dimensional case shown here, this would be a switch from a 12° to a 24° eye displacement site or a similar shift in final orbital position. Such a shift might occur via inactivation of a particular portion of the saccade map in the MRF that projects to either the SC or the NRTP.

To utilize the better understood SC saccade map, assume that “quasi-visual” and “build-up” neurons of the SC provide a desired displacement signal (ΔE) to the medium lead burst neurons in the PPRF (Mays and Sparks 1980; Munoz and Wurtz 1995a,b). Then, MRF RTLLBNs with presumed inhibitory projections to a region of the SC that mediates large, upward movements would be inactivated. This loss of inhibition would shift the distribution of activity on the collicular map toward the medial (upward eye movement) and caudal portion of the SC, thus generating upward, hypermetric saccades.

An alternative route for shifting model input utilizes projections from the MRF to the NRTP (Crandon and Keller 1985; Edwards 1975; Edwards and Olmos 1976; Lefevre et al. 1998; Suzuki et al. 1994). Inactivation of the fastigial nuclei, to which the NRTP projects, generates a significant degree of ipsilateral hypermetria and contralateral hypometria (Robinson et al. 1993; van Opstal et al. 1996). Although no changes in latency were observed after muscimol inactivation of the fastigial nuclei, the inaccuracies of saccade endpoints and curvature of saccade trajectories were similar to those found after caudal MRF inactivation. This suggests that inactivation of the caudal MRF RSLLBNs could shift activity in a NRTP → Cerebellum (fastigial nuclei) → PPRF and/or → SC loop in a similar way as in the proposed MRF → SC pathway (Lefevre et al. 1998; Quaia et al. 1999). In either scenario, changes in model inputs would preserve the relationships between amplitude and duration, as well as amplitude and velocity (Fig. 16A, new input) (Robinson et al. 1993). Hypermetric saccades with a normal amplitude/velocity relationship but longer duration for a given amplitude were noted after one injection (g0217, Figs. 4 and 10). Longer saccade duration would not be observed after a shift in model input. Moreover, shifts in saccade inputs could not account for the goal-directed nature of postinjection spontaneous saccades.

Inactivation in the feedback pathway of either model would also produce saccade hypermetria (Hyper #2). In this schema we propose that the build-up neurons in the intermediate and deep layers of the SC in conjunction with MRF RTLLBNs directly participate in the local feedback pathway (i.e., are in the feedback loop) (Waintzman et al. 1991, 1996). We hypothesize that via reciprocal inhibitory connections between the MRF and the SC, a spatial integration of eye velocity could occur (RI, Fig. 1B). Although the idea that the SC is involved in the spatial integration of a velocity signal has been suggested previously (Lefevre and Galiana 1992; Optican 1994), recent data do not support a role for the SC alone as the resettable integrator for the displacement model. Muscimol inactivation of the SC produces curved saccade trajectories and hypometria, but not saccade hypermetria (Aizawa and Wurtz 1994, 1998; Quaia et al. 1998). Alternatively, MRF neurons could be a part of a velocity to position integrator that is not reset at the end of each saccade (i.e., part of the NI in the Eye Position model, Fig. 1A). In the latter case, MRF neurons should be sensitive to initial eye position. The eye velocity command signal (Vc) from the medium lead burst neurons in the PPRF would be channeled to MRF neurons, where it would be integrated to produce eye displacement (i.e., the Eye displacement model) or eye position (Eye position model) (Benevento et al. 1977; Buttner-Ennever and Buttner 1988; Edwards 1975; Horn et al. 1994; Scudder et al. 1996). Loss of the resettable integrator or damage to the velocity to position integrator of Robinson results in modest lengthening of saccade duration, commensurate reduction in the velocity of postinjection saccades, and increased saccade amplitude (Fig. 16A, reduced feedback). In fact, saccade duration was moderately increased after the muscimol injection of g0217, with preservation of the amplitude/velocity relationship (Figs. 5, A and C, and 10C). Thus effects...
on saccades following injection g0217 could be explained by a reduction in the gain of the local feedback pathway of either model (Hyper #2, in Figs. 1, A and B, and 16A, reduced feedback, solid line).

However, for three other injections, saccade hypermetria was associated with normal or shorter saccade duration and either normal or higher saccade velocity (c0521, c0419, and c0416). Explanation of increased saccade velocity (over control) necessitated modulation of another model parameter. Increasing the gain of the burst generator causes saccades to be executed faster while accurately reaching their goal (Fig. 16B, i.e., the burst generator is in the local feedback loop). This condition was not observed in our data. In short, we were able to replicate saccade hypermetria and increased velocity in both the Eye Position and Eye Displacement models only by simultaneously increasing the gain of the burst generator while reducing the feedback of current eye position or displacement (Fig. 16C). Note that the simulated saccade amplitude was hypermetric and its velocity was higher, but saccade duration was slightly reduced compared with the amplitude matched control movement. We hypothesize that a combination of saccade hypermetria and increased velocity might occur if muscimol inactivation affected both RTLLBNs (feedback) and caudal RSLLBNs (feed-forward) in the MRF. Loss of the RTLLBNs would severely impair the feedback of current eye position or displacement activity to the SC. Impaired RSLLBNs output could remove inhibition on NRPo and NRPC neurons, thus increasing the number of spikes produced by medium lead burst neurons for a given amplitude saccade.

In summary, reduced feedback would account for the increased amplitude of saccades seen after all injections. However, we must hypothesize an increase in the gain of the pontine burst generator in the PPRF to account for the increased saccade velocity demonstrated in three other injections.

Mechanisms for the generation of square-wave jerks

Two questions arise regarding the generation of the repetitive macrosaccadic square-wave jerks. First, how do the two

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**FIG. 16. Simulation of changes in the feed-forward or feedback pathways for eye movement control.**

A: 2 conditions are shown. A shift in the input from a small (12°) to a large (24°) saccade region (Hyper #1, -- --). Inactivation of the feedback pathway in either the eye position or eye displacement models produces hypermetric saccades (Hyper #2). A reduction of 50% in the gain (top panel) of the feedback path is illustrated (---). Note that saccade peak velocity (bottom panel) is unchanged, but saccade duration is increased. This was the case for injection g0217 (see Fig. 10C). B: inactivation of the feed-forward pathway that normally provides inhibition to the burst neurons in the PPRF is hypothesized. This would cause an increase in the gain of PPRF excitatory burst neurons. As a result there is a marked increase in saccade velocity (bottom panel) without a change in saccade amplitude (top panel). C: a combination of the above 2 effects produces a marked saccade hypermetria (left panel), a moderate increase in saccade velocity (right panel) and intermediate effects on saccade duration (i.e., it remains the same or slightly reduced). These were the effects observed in injections c0416, c0521, and c0419.
models generate these characteristic movements? Second, can either of the models produce goal-directed movements? The answer to the first question turns out to be straightforward. Generation of saccade hypermetria from a reduction in the gain of feedback in each model leads to an unresolved motor error ($e_m$). If the model is stable, then oscillations around the final position should slowly die away and the eye will come to rest at the desired, eccentric location ($\Delta E$ or $e_d$). We observed this behavior for both models with the proper selection of feedback gain (i.e., ~60% of normal gain for both models Fig. 17A, 0.6 gain). When feedback was reduced by ~50% in the eye displacement model, the eye continued to oscillate around the final desired eye position without any decay (Fig. 17A, 0.5 gain). With further reductions in feedback gain, both models could be made very underdamped, in which case the oscillations increased, completely destabilizing the system (Fig. 17A, 0.25 gain).

The answer to the second question (goal-directed saccades) was addressed by reducing feedback (i.e., at the Hyper #2 location) by 50% and asking each model to execute saccades from different initial positions to the same final position. Neither model could generate goal-directed saccades in this

FIG. 17. Simulation of macrosaccadic square-wave jerks. A: a decrease in the gain (50%) of the local feedback pathway (Hyper #2, Fig. 1) in either the Eye Displacement or the Eye Position models results in saccade hypermetria, and then persistent oscillation of the eyes around the intended final eye position (— —). A small increment in this gain (60%) in each model allowed the error to decay over time so that the eyes eventually reached the goal (—). A large reduction in gain (25% of normal) resulted in unstable oscillations (— — —). A reduction in feedback gain at the Hyper #2 location of either model could not simulate orbital position effects. B: placement of a small delay (110 ms) in the portion of the feedback pathway of the Robinson model leading to SJ2 (Hyper #3) also produce macrosaccadic square-wave jerks (—). However, different from the simulation in A, these movements would often reach a specific orbital position after a small overshoot (saccade 3). Open loop conditions (delay of 50 s) produced hypermetric, unstable oscillations (— — —, saccades 5 and 6). — — —, target; •••, normal, control eye movements.
scheme (data not shown). Rather, each produced saccades that oscillated around the final position without actually reaching the final position. With appropriate choices of feedback gain, the oscillations for each model would eventually die away, and the final position would be achieved (similar to the 0.6 gain shown in Fig. 17A), but these are not goal-directed movements. On the other hand, goal-directed saccades could be produced in some circumstances by careful manipulation of the Eye Position model (Fig. 1A). By placement of a moderate delay (110 ms) in the second limb of the feedback loop directed to summing junction 2 (SJ2, Fig. 1B, Hyper #3, del) macrosaccadic, hypermetric, square-wave jerks could be generated (Fig. 17B, Hyper #3, solid line). Feedback gain in the portion of the feedback loop reaching SJ3 was left intact (i.e., it was 1.0). The interpretation of this result was that the local feedback loop (SJ3) would control execution of the proper initial saccade to the desired orbital position (Fig. 17B, 1). In the meantime, without proper feedback of current eye position to SJ2, retinal error (Rret) would fall to zero without a commensurate rise in E’. This would result in a sudden drop in e_4 as if a desired eye position of zero was needed, and a saccade in the opposite direction would be generated (Fig. 17B, 2). However, 110 ms after this “return” saccade ended, the original current eye position signal (E’) would suddenly arrive at SJ2. This new “current eye position” would be summed with the residual retinal error (Rret) remaining after the return saccade. Again a new desired orbital position (e_d) would be produced, initiating a third movement (after a normal refractory period) of similar amplitude and direction (+ a small error) toward the original goal (Fig. 17B, 3). The small “overshoot” seen at the end of this movement (Fig. 17B, 4) is the result of the previous retinal error, which disappears quickly as the new residual retinal error goes to zero. Note the difference between this tracing and that obtained with changes at Hyper #2 (Fig. 17A). Oscillations around the goal (with the exception of the overshoot) were abolished. These movements, albeit one dimensional in the model, are very similar to the repetitive goal-directed, macrosaccadic square-wave jerks we observed after inactivation of the caudal MRF. In open loop conditions (i.e., via placement of a long delay in the feedback loop), repetitive saccades were generated, but they did not land on the orbital position goal and became progressively larger in amplitude (Fig. 17B, dashed-dotted line, 5 and 6). Again similar, but not exactly like the square-wave jerks observed experimentally. In conclusion, both models can generate hypermetric, macrosaccadic square-wave jerks, but only the Robinson model could be adapted to generate repetitive goal-directed movements. Clearly new models of higher dimensionality and increased sophistication are needed to more fully characterize the oculomotor behavior found following muscimol inactivation of the caudal MRF.

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