Eye Movement Deficits Following Ibotenic Acid Lesions of the Nucleus Prepositus Hypoglossi in Monkeys II. Pursuit, Vestibular, and Optokinetic Responses

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Kaneko, Chris R. S. Eye movement deficits following ibotenic acid lesions of the nucleus prepositus hypoglossi in monkeys. II. Pursuit, vestibular, and optokinetic responses. J. Neurophysiol. 81: 668–681, 1999. The eyes are moved by a combination of neural commands that code eye velocity and eye position. The eye position signal is supposed to be derived from velocity-coded command signals by mathematical integration via a single oculomotor neural integrator. For horizontal eye movements, the neural integrator is thought to reside in the rostral nucleus prepositus hypoglossi (nph) and project directly to the abducens nuclei. In a previous study, permanent, serial ibotenic acid lesions of the nph in three rhesus macaques compromised the neural integrator for fixation but saccades were not affected. In the present study, to determine further whether the nph is the neural substrate for a single oculomotor neural integrator, the effects of those lesions on smooth pursuit, the vestibulo-ocular reflex (VOR), vestibular nystagmus (VN), and optokinetic nystagmus (OKN) are documented. The lesions were correlated with long-lasting deficits in eye movements, indicated most clearly by the animals’ inability to maintain steady gaze in the dark. However, smooth pursuit and sinusoidal VOR in the dark, like the saccades in the previous study, were affected minimally. The gain of horizontal smooth pursuit (eye movement/target movement) decreased slightly (<25%) and phase lead increased slightly for all frequencies (0.3–1.0 Hz, ±10° target tracking), most noticeably for higher frequencies (0.8–0.7 and ~20° for 1.0-Hz tracking). Vertical smooth pursuit was not affected significantly. Surprisingly, horizontal sinusoidal VOR gain and phase also were not affected significantly. Lesions had complex effects on both VN and OKN. The plateau of peri- and postrotatory VN was shortened substantially (~50%), whereas the initial response and the time constant of decay decreased slightly. The initial OKN response also decreased slightly, and the charging phase was prolonged transiently then recovered to below normal levels like the VN time constant. Maximum steady-state, slow eye velocity of OKN decreased progressively by ~30% over the course of the lesions. These results support the previous conclusion that the oculomotor neural integrator is not a single neural entity and that the mathematical integrative function for different oculomotor subsystems is most likely distributed among a number of nuclei. They also show that the nph apparently is not involved in integrating smooth pursuit signals and that lesions of the nph can fractionate the VOR and nystagmic responses to adequate stimuli.

INTRODUCTION

The oculomotor neural integrator is a mechanism that converts velocity-coded commands from the various oculomotor subsystems into the eye-position-coded discharge of motoneurons. The integrator is thought to tonically drive ocular motoneurons to maintain accurate eye position after eye movements (Robinson 1968). In addition, for eye position to remain accurately on target, integrated eye movement commands from different oculomotor subsystems must be congruent. For example, when a person is running to catch a thrown object, the vestibulo-ocular reflex (VOR) stabilizes the image of the object to compensate for movement of the head, and it does this in precise coordination with the pursuit and saccadic eye movements that track the object during its flight. Robinson (1968, 1989) surmised that the most efficient way to standardize the signals from the various oculomotor systems was to have a single common neural integrator that supplies a consistent position drive to motoneurons.

The nucleus prepositus hypoglossi (nph) has been proposed (Baker et al. 1981) as the unique neural structure that subserves the integrative function for all horizontal eye movements. Originally based on insightful conjecture, this proposal since has been supported by a number of findings. Physiological studies in alert animals showed that the discharge of nph neurons is appropriate for integrating velocity signals to position-related discharge (i.e., tonic and burst-tonic discharge) (Lopez-Barneo et al. 1982; McFarland and Fuchs 1992). The nph also has the appropriate anatomic connections to mediate integration (see McCrea 1988 for review). For example, in monkeys, the rostro-lateral margin of the nph, called the marginal zone (Belknap and McCrea 1988; Langer et al. 1986), supplies the output from the nph to the abducens nuclei. These findings are consistent with the nph being a portion of the horizontal integrator but not necessarily the sole nucleus. The most compelling evidence that the nph is the only horizontal neural integrator comes from a variety of lesion studies in which transient inactivation of the nph was correlated with loss of horizontal integrator function for all horizontal eye movements (Cannon and Robinson 1987; Cheron and Godaux 1987; Cheron et al. 1986).

In contrast, other evidence has hinted that the integrative process may be more complex. First, other structures can affect neural integration (for discussion, see Fukushima et al. 1992). For example, destruction of the flocculus and paraflocculus leads to a shorter integrator time constant (Zee et al. 1981). The shorter time constant is not easily attributed to floccular effects on nph because the flocculus does not project to the nph in monkeys (Langer et al. 1985). Similarly, injections of neuroactive substances such as muscimol, γ-aminobutyric acid (GABA), and bicuculine into the central medial vestibular
nucleus without impinging on the nph, affected gaze stability and vestibular imbalance just as they did when injected into the nph (e.g., Metten et al. 1994; Straube et al. 1991). Second, eye-position sensitivity is not the same for each of the oculomotor subsystems. If there was a single common integrator, one might expect a constant relationship (see discussion in Godaux and Cheron 1993). For example, position sensitivity for motoneurons during fixation varies with stimulation frequency and from motoneuron to motoneuron but, on average, is about half of what it is during smooth pursuit or the VOR (e.g., Fuchs et al. 1988). In fact, several premotor areas that project directly to motoneurons have been shown to have varying position sensitivities depending on the eye movement (nph: McFarland and Fuchs 1992; medial vestibular nucleus: Scudder and Fuchs 1992; interstitial nucleus of Cajal: Kaneko and Fukushima 1998; but see Godaux and Cheron 1996).

Third, recent evidence has shown that the ability to maintain steady fixation in the dark postsaccadically can be affected independently of the gain or metrics of saccades as a result of nph lesions (Kaneko 1997). Last, a number of other structures have been identified as integrators in the oculomotor system. For example, the integrator for vertical VOR certainly is distributed between the vestibular nuclei and the interstitial nucleus of Cajal (for discussion, see Fukushima and Kaneko 1995). Similarly, the velocity storage integrator is associated with the nodulus and uvula (e.g., Wearne et al. 1998). That integrator stores vestibular and optokinetic velocity and is separate from the velocity-to-position integrator.

In addition to the evidence contradicting the hypothesis of a single neural integrator, there are limitations to the evidence supporting it. The strongest evidence for a single horizontal integrator in the nph comes from studies that involved neurotoxic lesions, but in all those studies, the nph lesions were impermanent and the oculomotor deficits were transitory (Cannon and Robinson 1987; Cheron and Godaux 1987; Cheron et al. 1986). As argued previously (Kaneko 1997), the ibotenic acid used in these studies probably undergoes rapid in vivo decarboxylation to muscimol (e.g., Curtis et al. 1979), a known GABA mimic that is effective in nanomolar concentration and also inhibits the uptake of GABA. Thus one might expect short-term stimulation of GABA receptors after the injection of ibotenate, and this might result in much more diffuse effects than a punctate lesion would cause. To address these issues, the present study focused on the permanent oculomotor deficits that resulted from documented lesions of the nph.

A previous report from this laboratory showed that the ability to maintain stable gaze in the dark can be affected severely, permanently, and immediately by even very small lesions of the monkey rostral nph (Kaneko 1997). These changes occurred without the concomitant alteration of saccadic gain or metrics that would be expected if the saccadic feedback integrator (Robinson 1975) also had been damaged. Thus at least the feedback integrator must be ascribed to structures other than the nph, whereas the nph appears to function primarily for fixation. In the present study, the effects of rostral nph damage on smooth pursuit, the VOR, vestibular nystagmus (VN), and optokinetic nystagmus (OKN) were quantified in the same animals used in the previous study in an attempt to determine whether the nph is the single neural integrator for horizontal eye movements. Preliminary portions of this work have been published in abstract form (Kaneko 1992; Lambert and Kaneko 1995).

METHODS

The methods used in this experiment are similar to those described recently elsewhere (Kaneko 1996, 1997). Subjects were the three juvenile, male rhesus macaques (Macaca mulatta) used in the previous study (Kaneko 1997). A scleral search coil (Fuchs and Robinson 1966), a recording chamber, and stabilization lugs were implanted under aseptic surgical conditions. The monkeys were trained to track a moveable, back-projected laser spot for food reward. All experiments were performed in strict compliance with the Guide for the Care and Use of Laboratory Animals (DHEW Publication NIH85-23, 1985) and exceeded the recommendations from the Institute for Laboratory Animal Resources and the Association for Assessment and Accreditation of Laboratory Animal Care International. Specific protocols were approved by the local Animal Care and Use Committee of the University of Washington (ACC 2602-01).

After surgery and training, the nph was mapped and punctate lesions were placed in a portion of the nph by the use of pressure injections of ibotenic acid (Kaneko 1997). Lesions were confirmed histologically in normal Nissl-stained material or immunohistochemically by staining for glial fibrillary acidic protein (GFAP) in astrocytes (O’Callaghan 1991). Staining was done with a modification of standard peroxidase-antiperoxidase immunohistochemistry (Sternberger 1986) and commercially available rabbit anti-GFAP (Sigma).

Eye-movement data were collected as early as a few minutes after the injection and periodically thereafter at increasingly longer intervals of hours, days, and weeks until there were no further changes in the parameters of the eye movements. The analysis of saccades and drift in the dark has been described previously (Kaneko 1997). Smooth pursuit was elicited by requiring the animal to track a small (−0.5°) red spot projected from a laser diode via mirror galvanometers onto a white background that was attached temporarily to the optokinetic drum that surrounded the animal. Data were collected during both horizontal and vertical, ±10° sinusoidal movements at 0.3, 0.5, 0.7, and 1.0 Hz. VOR was evoked by whole-body oscillation with the animal’s head affixed rigidly to the superstructure of the animal chair. Oscillations were ±10° at 0.1, 0.25, 0.5, 0.7, and 1.0 Hz for horizontal (yaw) and vertical (pitch) rotation. The animals also were tested at 2.0-Hz yaw, but the apparatus was incapable of pitch at that frequency and excursion. Vestibular and optokinetic eye movements were evoked by continuous rotation in the horizontal plane. For VN, the animal and chair were rotated en bloc, whereas OKN was produced by rotating an optokinetic drum with a Julesz pattern that surrounded the animal. The apparatus did not allow continuous rotation in the vertical plane. VN data were collected for velocity trapezoids that had constant rotational plateaul velocities usually of 40 and 80°/s in both clockwise (cw) and counterclockwise (ccw) directions. The plateau velocities were maintained until eye velocity returned to zero and then the chair was stopped. Acceleration and deceleration portions of the trapezoids were constant at 500 deg·s−1·s−1. OKN was elicited by accelerating the drum to 30, 60, 90, or 120°/s in the dark and then turning on the drum lights. When optokinetic nystagmus had reached and maintained a steady velocity for ≈100 s, the lights were turned off and optokinetic afternystagmus (OKAN) was allowed to decay to zero velocity (however, see RESULTS). Both cw and ccw drum rotations were used. Initial nystagmus data from monkeys M and Z proved quite variable, so subsequent monkeys were kept alert with small doses (usually 0.25 mg/kg) of amphetamine sulfate (Cohen et al. 1977) given just before nystagmus was tested. (Amphetamine sulfate was not administered until after all other eye movements had been characterized.) This procedure was adopted after lesion 4 in monkey M and for all the nystagmus testing in monkey R.

Smooth pursuit and VOR were analyzed as described recently (Kaneko and Fukushima 1998). After eye and chair or target signals
were digitized at 1.0 kHz, each cycle for a particular stimulus frequency was divided into 512 equal-duration bins and presented on a computer screen so saccades or segments of poor tracking could be marked manually and deleted. The resulting segments were fit with a sinusoid using a least-squares method (Schor 1973). At least 10 cycles were used for each fit.

Nystagmus was analyzed with the aid of another interactive program. Previous programs from this laboratory calculated velocity from the digitized eye-position signal, but in the current study, eye velocity was calculated with the use of an analog circuit that limited noise in that signal. The linear-phase velocity circuit consisted of a five-pole Bessel filter with a cutoff frequency of 20 Hz for velocities <100°/s and 200 Hz for those greater than that. The circuit had a constant delay (7 ms for high gain scale and 20 ms for the low), which was ignored in the following analysis. The output of the circuit was calibrated with the use of triangle wave inputs from a function generator the rate of change of which corresponded to stimulus velocity (e.g., 40 and 80°/s for VN), and the results of the analysis were scaled appropriately for each velocity. Both eye position and velocity, along with either chair velocity or drum illumination signals, were digitized at 1.0 kHz. Quick phases were deleted from the data in an editing process involving the use of adjustable velocity and acceleration criteria that were tuned to eliminate both the quick phase and a selectable number of points on either side of the quick phase (usually ±2.5). This process effectively eliminated any contribution of transient components of the quick phases to the estimation of slow phase eye velocity (cf. Rey and Galiana 1993). The remaining data were smoothed (usually 5-point running average) and then presented in two windows on the computer screen. In the expanded time-base view, the experimenter marked the rapid events, such as the initial VOR response and chair movement of OKN or the light-on and the initial smooth eye movement of OKN. In the compressed view (compressed to 600 points by averaging all remaining points in each of 600 equal duration bins), the experimenter marked the plateau and decay of VN or the charging and discharging phase and the steady-state velocity portions of OKN. Exponential time constants for the charging and discharging phases of OKN and the decay portions of VN were calculated with the use of a least-squares fit of the natural log transform using the equation \( V(t) = V_f (1 - e^{-\tau t}) \) where \( V(t) \) is the eye velocity at time \( t \), \( V_f \) is the final eye velocity, and \( \tau \) is the time constant. If the fit was not significant (\( P > 0.01 \)), it was not included in the averages or the figures. If a quick phase occurred at the time of the peak velocity when the light or chair was turned on or off, the peak velocity immediately preceding the quick phase was substituted.

**RESULTS**

The lesions that resulted from the ibotenic acid injections have been documented in detail (Kaneko 1997). Monkey Z received a single large (~1.5 μL) injection that resulted in obvious, long-lasting deficits in gaze stability in the dark and more transient saccadic deficits. The animal also exhibited a complex array of OKN changes but no apparent changes in either smooth pursuit or VOR. Because the fixation, saccade, and OKN deficits were so pronounced but pursuit and VOR were seemingly normal, pursuit and VOR were not tested extensively in the first two animals (monkeys Z and M). With monkey R, which received serial, punctate lesions that could provide a basis for comparison with the progressive eye-movement deficits, minimal effects were substantiated through extensive documentation of changes in the VOR and pursuit.

Monkey Z’s injection was centered just caudo-ventral to the left abducens nucleus and resulted in nearly complete destruction of the left nph and marginal zone, signs of damage (i.e., gliosis) to the medial edge of the left medial vestibular nucleus (mVN), and frank mechanical damage (~2 mm height × ~1.25 mm width × ~2 mm length) to the reticular formation and medial longitudinal fasciculus just ventral to the nph/mVN owing to the injected volume of fluid (Fig. 2 in Kaneko 1997). Monkey M had five small (180- to 500-ml) injections that resulted in obvious but subtotal damage to the rostral nph and marginal zone. The first two injections (180 and 200 mL) were on the left and right sides, respectively, and resulted in minimal transient oculomotor effects and barely discernible gliosis. Therefore the volume was increased for injections 3 and 4, which were on the right side ~1.25 mm caudal to the caudal edge of the abducens at the center of the caudal marginal zone. Both those and injection 5, which was on the left centered within the marginal zone, resulted in dense gliotic staining of ~0.75 mm in diameter (Fig. 3 in Kaneko 1997). Monkey R received eight sequential injections of 350–700 nL; these injections (4 on each side) completely destroyed the right marginal zone and rostral nph and caused substantial damage to the rostral half of the nph on both sides. The maximal extent of damage was estimated by GFAP immunohistochemistry (Fig. 4 in Kaneko 1997).

The strategy was to make a lesion and measure resultant eye-movement deficits. For monkeys M and Z, the eye movements were quantified during subsequent testing at progressively longer intervals until they did not seem to be changing further. Then the nph was remapped and the monkey received another injection. With this approach, it was possible to titrate the nph damage against the oculomotor dysfunction and eventually correlate the reconstructed, permanent lesions with the long-term eye-movement deficits. The procedure did not permit repeated measures during a given testing session because the battery of tests was quite extensive and the monkeys would not work for the protracted period required for repeated testing. This meant that for a few measures, as detailed in the following text, statistical tests could not be performed because estimates of the variability of the measurements were not available for an individual test day and the data across testing sessions were not stationary. The experimental design also allowed for adaptation as well as recovery from the transient effects of the injections so that the values reported here necessarily reflect both the deficit associated with the lesion and the adaptive properties of the system invoked to compensate for the damage.

**Smooth pursuit eye movements**

Although preliminary observations on monkeys Z and M (tested with sinusoidal target movement presented at 0.5 Hz, ±10°) suggested no obvious change in pursuit capabilities, damage to the nph in monkey R did result in small but consistent decreases in the gain of sinusoidal smooth pursuit (amplitude of the sinusoidally fit eye position/fit target position) and increases in phase lead that were obvious only at higher tracking velocities. Initial assessment of the ability to track ±10° sinusoidal target movements at 0.5 Hz, like the qualitative assessment in monkey Z, showed no significant effect on phase (phase lead/lag within ±3° of the target) for monkey M. Similarly, the gain of smooth pursuit after each of the first two lesions in monkey M remained unaltered even though those lesions caused significant, permanent impairment of the ability to maintain peripheral eye position in the dark (Kaneko 1997). Because assessment of
horizontal and vertical pursuit indicated that minor changes in horizontal pursuit were within the normal variability of pursuit, it was not measured after each of the last three lesions in monkey M. Contrary to the preliminary conclusion based on these observations of pursuit tracking at a stimulus frequency of 0.5 Hz (Lambert and Kaneko 1995), a more detailed analysis of a wider range of frequencies in monkey R suggests that there are indeed subtle deficits associated with more extensive nph damage. These deficits are most clear for higher tracking velocities (1.0 Hz, $671^\circ$).

Figure 1 shows the gain and phase for horizontal smooth pursuit at four frequencies relative to target position. Prelesion performance is represented by the symbols near the ordinate and the eight clusters of points represent the measurements taken after each lesion. Gain decreased slightly over the course of the eight lesions but not significantly ($P > 0.01$, t-test between normal and final gain indicated by SD error bars) compared with prelesion values. The decrease was particularly evident for higher frequency sinusoidal target movements. At 1.0 Hz, for example, gain declined from normal values of $\sim 0.8$ to as low as 0.6 immediately after the last lesion but recovered to 0.8 by day 97 (Fig. 1, top, rightmost dot). Likewise, subtle phase changes were evident only at the higher frequencies (Fig. 1, bottom). When the target was oscillated at 0.7 and 1.0 Hz, the eye normally lagged the target by $\sim 16^\circ$ before the first lesion, but there was a gradual phase shift over the course of the lesions, concluding with a phase lead of $\sim 3^\circ$ after the eighth lesion. This trend was not seen at the lower frequencies and actually was reversed for tracking at 0.3 Hz, where the initial phase lead of $\sim 7^\circ$ decreased only slightly over the course of the lesions. Owing to the small variability in the measurements (see error bars), the increasing phase lead for higher frequencies was statistically significant ($P < 0.01$, t-test). For clarity, the insets (bracketed by open arrows) show typical gain and phase on a fourfold expanded time scale (same gain scale) for data after the fourth injection. The inset shows that the gain dropped immediately after a lesion, but recovered over the first few days postlesion. Similar plots (not shown) of gain and phase relative to target velocity showed identical changes over the course of the series of injections, indicating consistent dynamics across the tested tracking frequencies.

The nph is thought to be concerned only with horizontal neural integration, but neurons with vertical on directions can be recorded in that region (Kaneko 1997). To check whether nph damage affected vertical smooth pursuit, we also quantified vertical pursuit performance (Fig. 2). Although there was more scatter in the data owing to the monkeys’ inherently
poorer tracking ability and asymmetry for vertically moving targets, there was no indication of the long-lasting changes in gain seen during horizontal pursuit. There was an initial movement toward 0° phase (i.e., eye and target aligned) over the first two lesions, similar to that seen for horizontal pursuit (cf. Fig. 1). This result suggests that these initial changes in phase may be due to learning and/or predictive adaptation by the animal, the better to match target and eye position. Recall that the animals were rewarded for maintaining eye position near target position, so improved matching might be expected. Comparison of the data in Figs. 1 and 2 shows that the effects of nph damage were specific to horizontal pursuit: whereas the animals consistently showed an initial drop in pursuit gain during horizontal pursuit after an injection (Fig. 1, filled arrows), no such drop occurred during vertical pursuit (Fig. 2, gain inset).

Sinusoidal VOR

As with pursuit, sinusoidal VOR in the dark was catalogued only for monkey R because preliminary tests in monkeys Z and M suggested that there were no consistent changes after ibotenic acid injections. The results of the more complete analysis of monkey R’s data confirm the initial observations in the first two animals. As shown in Fig. 3, there were only small, inconsistently statistically significant changes in gain and phase at higher frequencies of rotation. The data are plotted relative to chair (= head) position. There was an increase in variability of the gain as indicated by the error bars, which are about the size of the symbols for normal values on the ordinate but much larger for final values after lesion 8. Gain during rotation at 0.25, 1.0, and 2.0 Hz decreased slightly (0.05–0.11) but significantly (P < 0.01, t-test), whereas gain during rotation at 0.1 and 0.5 Hz did not change significantly. Phase also changed slightly (6–9°) and inconsistently. There was a significant (P < 0.01, t-test) shift in phase lead (~6°) at 0.5 Hz (triangles) but a significant shift in phase lag (6–9°) at 1.0 and 2.0 Hz. If the vestibular velocity to position integrator resided in the nph, one would expect a decrease in gain and a phase shift toward velocity (i.e., ~90° lead) especially at lower stimulus frequencies. If anything, eye position was more nearly exactly compensatory for chair position (i.e., exactly 180° out of phase) after the last of eight lesions (rightmost clusters in Fig. 3). Phase lag showed a transient increase after the first lesion, but it recovered immediately and remained near initial values.

VOR data also were collected during vertical sinusoidal pitch oscillation in the dark (Fig. 4). Interestingly, there were some apparent transient changes in the gain and phase of vertical VOR. Gain seemed to decrease slightly (from ~0.9 to ~0.8) after the first lesion. Phase decreased from ~180° before lesion 1 to ~195° after lesion 4 and then recovered to near normal levels over the course of lesions 5–8. The slight (~15°) transient variations in vertical VOR phase over the course of the injections are apparent (Fig. 4, bottom) when compared with the relatively unchanged (~5°) horizontal VOR (Fig. 3, bottom). Unlike the pursuit data, vertical VOR scatter was about the same as that for horizontal VOR.
FIG. 3. Horizontal sinusoidal vestibular ocular reflex (VOR) in the dark. Time course of changes in VOR as in Fig. 1. Responses were quite consistent and changes were minor: a small, transient initial change in phase, increased variability of gain (error bars) after lesions 7 and 8, and a modest, inconsistent decrease in gain overall (see text). Top: gain. Bottom: phase. Error bars, SD. – – –, average prelesion response.

FIG. 4. Changes in vertical VOR. Conventions as in Fig. 3.
Vestibular nystagmus

In sharp contrast to the consistency of the response to sinusoidal oscillations, continuous horizontal rotation revealed a number of changes in vestibular function associated with nph damage. Monkey Z, which received the large unilateral nph lesion, showed dramatic asymmetry in the VN response. Chair acceleration to a constant rotational velocity followed by deceleration to rest is called a velocity trapezoid because the profile of the velocity over time is trapezoidal. Such stimuli normally result in an initial per-rotatory nystagmic response to the acceleration, but eye velocity returns to zero during the constant-velocity rotation. The symmetric postrotatory response observed when the chair decelerates is the mirror of the acceleration response (see Fig. 5A). In monkey Z, the nph lesion caused VN to be disrupted completely for rotations toward the lesion side. The initial per-rotatory and plateau phases were similar to the normal (Fig. 5A) response but the slow eye velocity did not decay during the continuous rotation for periods as long as 150 s and there was very little postrotatory response (see Fig. 3 in Kaneko 1992). A step in eye position like that described by Cannon and Robinson (1987) never was observed in response to a step of chair velocity after these unilateral injections.

The changes associated with smaller, sequential punctate nph lesions were not as catastrophic and provided some clues about which components as well as which oculomotor subsystems were affected similarly by nph damage. The data are illustrated most clearly for monkey R, which received eight separate injections (4 on each side), but the general trends were identical to those obtained after the first few injections in monkey M. The various components of the vestibular response are illustrated in Fig. 5, which compares data taken during 40°/s cw (rightward) rotation in the dark before injections began (Fig. 5A) and on day 97 after lesion 8 (Fig. 5B). The per-rotatory VOR to the ramp of chair velocity (initial) was followed by a prolonged, maintained period of compensatory eye velocity (plateau) and eventually began to decay exponentially \( \tau \) to reverse the direction of the slow eye-velocity as vestibular afternystagmus. Chair deceleration resulted in a mirror image postrotatory response (Fig. 5A, top right).

Lesions of the nph produced reductions in the initial response, the duration and peak velocity of the plateau, and the
The postrotatory changes were completely analogous to the per-rotatory changes, and there was no obvious difference between the ccw and cw responses. An example of the changes with successive injections is illustrated in Fig. 6, which shows that the plateau duration increased slightly initially as a result of the first few injections but eventually decreased to below normal values, lasting just a few seconds after lesion 8. Not all metrics of vestibular nystagmus showed the same pattern of alterations. Changes in the time constant of the decay in slow eye velocity (Fig. 5A) showed a similar initial increase (Fig. 7) but then returned to normal or near-normal values during the course of the last injections, although postrotatory (Fig. 7, bottom) responses were slightly more affected than per-rotatory (Fig. 7, top).

In monkey M, which received five sequential lesions, the effects were less severe than those seen in monkey Z and similar to those illustrated for monkey R except that the estimated time constant was quite variable. This difficulty was resolved by the use of amphetamine, which produced a consistent level of alertness during the testing (see METHODS) and greatly improved the consistency of the response: the correlation coefficients of the exponential fits for 80°/s cw rotation increased from ~0.6 without amphetamine to ~0.9 with pretreatment. Statistically insignificant exponential fits virtually were eliminated for all stimulus velocities for both OKN and VN. Even though the variability was higher initially, the time course of changes in all VN metrics for monkey M was identical to the results after the analogous first few injections in monkey R (Figs. 6, 7, and 9) except for two minor differences. The scatter in VN time constant was larger after lesions 1–4, reaching higher maximum values (80 vs. 50 s) before recovery after lesion 5, and the absolute value of the initial jump in eye velocity was ~10°/s lower than that for monkey R.
Optokinetic nystagmus

To characterize and quantify any changes in OKN associated with lesions of the nph, we measured the various components of the response as described in METHODS. Figure 8A shows a typical example of prelesion OKN in monkey R at a drum velocity of 60°/s. The fast rise (jump, Fig. 8A, top) when the drum lights were turned on (Fig. 8A, 2nd trace) was followed by a more gradual build-up (charging) of slow-phase eye velocity until a plateau (steady state) was reached. When the light was turned off, OKAN began with a rapid decrease in the slow eye velocity (drop) and continued with a more gradual decay (discharging) toward zero (– – –) to peak OKAN. Peak and post-OKAN responses are not reported because they were small and inconsistent. Both the charging and discharging were well fit by a single exponential (see METHODS) and thus were characterized by their time constants.

After the large injection in monkey Z, drum rotation toward the side of the injection failed to elicit discernible OKN (Fig. 8C, top) even several weeks after the injection. Slow eye velocity did not rise consistently above zero (– – –) during drum rotation. This deficit was observed for all four tested drum velocities on each of several separate days of testing (average steady-state velocity <10°/s regardless of drum velocity) even though contralateral cw rotation tested at the same time produced a significant OKN response. Although VN in monkey Z was asymmetric, both per- and postrotatory cw responses were still evident, but OKN for ccw drum rotation apparently was compromised more severely.

To distinguish effects on individual components of the OKN response, we used much smaller quantities of ibotenic acid in monkeys M and R. The results of the most complete series in monkey R are shown in Fig. 9, which plots the changes in each oculomotor behavior (ordinate) over the series of lesions (abscissa). In contrast to the asymmetry resulting from the large injection in monkey Z, the series of punctate lesions in monkey R was associated with less severe deficits even though the rostral nph was completely compromised (Kaneko 1997). Figure 8B shows the OKN in response to the same 60°/s ccw drum rotation recorded 97 days after lesion 8. The initial jump was not much different from the normal response (Fig. 8A), but the steady-state velocity was lower and the charging and discharging seemed to be shorter (note different time calibrations). As for VN, the trends were similar in monkey M but the results showed much more day-to-day scatter, probably because amphetamine was not used until after the fourth injection. The consistency of the measurements was improved by the development of a new testing apparatus and the use of mirrors to restrict the field of view to the moving drum. The amphetamine

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**Fig. 8.** Optokinetic nystagmus. Records taken from the compressed screen (see METHODS) of the nystagmus analysis program (cf. Fig. 5). A: normal, prelesion response of monkey R during 60°/s ccw (animal leftward) drum rotation. Traces are (top to bottom) slow eye velocity, light on/off, and horizontal and vertical eye position. B: monkey R’s response to the same stimulus on day 97 after lesion 8 (vertical eye position omitted). C: response of monkey Z to the same stimulus 20 days after large (~1.5 μL) injection into left nucleus prepositus hypoglossi (nph). Second trace is drum velocity because a light signal was not available. Vertical eye position omitted. Note the lack of a consistent horizontal eye position response. Calibration is 60°/s for eye velocity, ±10° for eye position, and 5 s. – – –, 0 eye velocity or position.
The initial jump in OKN was reduced by lesion 8 compared with normal values, more so for 120°/s) in jump velocity then, at the third and fourth lesions, that for VN plateau duration (Fig. 6) for each of the drum triangles connect by dashed line) showed a pattern similar to metrics illustrated in Figs. 6 and 7. The initial jump in OKN followed one of the two patterns of change observed for VN the course of injections in the data for all of the oculomotor behaviors we tracked during the injections for the various metrics of OKN and summarizes and the new apparatus seemed to eliminate the periods of (see C). VN and OKN metrics that increased initially in association with nph damage and showed the highest variability; error bars = 1 SD.

and the new apparatus seemed to eliminate the periods of low-velocity drift that punctuated the nystagmic response and contributed to its variability.

Figure 9 shows the pattern of changes during the course of the injections for the various metrics of OKN and summarizes the data for all of the oculomotor behaviors we tracked during the course of injections in monkey R. The various OKN metrics followed one of the two patterns of change observed for VN metrics illustrated in Figs. 6 and 7. The initial jump in OKN velocity when the drum lights were turned on (Fig. 9B, gray triangles connect by dashed line) showed a pattern similar to that for VN plateau duration (Fig. 6) for each of the drum velocities tested. There was a slight increase (especially 90 and 120°/s) in jump velocity then, at the third and fourth lesions, initial jump velocity began to decrease below normal levels except for the slowest drum velocity (30°/s) and generally was reduced by lesion 8 compared with normal values, more so for ccw drum rotation. The initial jump in eye velocity of OKN was about the same for both monkeys M and R, unlike the higher values for monkey M’s initial jump in eye velocity of VN. The time constants derived from the exponential, least-squares fit of the charging phase (Fig. 8) of the OKN response showed the other pattern of change. They initially increased from normal values of 5–10 s to as high as 30 s (Fig. 9C, gray diamonds), but these values recovered over the course of the injections and ended around or above the normal levels (horizontal dashed lines) and showed much greater variability (note error bars). The values of the time constants were about half those of VN (Fig. 7) but otherwise showed very similar changes over the course of the injections (cf. Fig. 9C, boxed ×). Another similarity to VN is that the trend was the same in monkey M but the increase in time constants associated with the second and third lesions reached higher maximum values of 50–60 s for monkey M compared with 25–35 s in monkey R.

In contrast to the transient changes in time constant, the steady-state velocity (Fig. 9B, asterisks) decreased gradually and continually over the course of nph destruction from initial values of ~90% of the stimulus velocity. This decrease was particularly evident for the higher velocities. After steady-state OKN, there was an abrupt drop in eye velocity when the lights were turned off (Fig. 8). The changes in the drop in eye velocity (Fig. 9C, open triangles) did not mimic the changes in jump velocity (Fig. 9B, triangles). Instead the drop in velocity increased initially and remained elevated compared with baseline values or continued to increase slightly over the course of the injections (Fig. 9C, open triangles). There was little indication of the transient increase followed by recovery seen for OKN jump and the final values were higher, not lower, than normal values as they were for jump velocities. For all stimulus velocities and each injection, the variability in the response appeared larger than that seen for jump velocity. Finally, the changes in the discharging time constant (Fig. 9C, diamonds) did not parallel the charging time constant (Fig. 9B, dots). There was often a slight initial increase associated with the second and third injections, but the time constant returned to normal values and then became consistently shorter than normal values, reaching <10 s by the eighth lesion.

**DISCUSSION**

The results of this study show that progressive damage to the rostral nph of the monkey is associated with only very minor changes in sinusoidal smooth pursuit and the VOR but produces considerable fractionation of VN and OKN. The latter effects are not uniform but alter the subcomponents of the responses differentially (Fig. 9). Previous results showed that nph damage in the same animals was associated most immediately with partial failure of the fixation-holding integrator, manifest as incessant centripetal drift of peripheral gaze toward a null position in the dark but not with dysfunction of the putative saccadic feedback integrator (Fig. 9A) (Kaneko 1997). Because the time constant of centripetal drift was reduced drastically after even a single lesion, whereas all other possible integrator functions showed progressive incremental deficits, it is most likely that the rostral nph is the site of the saccadic velocity-to-position integrator that is responsible for maintaining stability of peripheral gaze (Baker et al. 1981; Cannon and Robinson 1987; Cheron and Godaux 1987). The very modest and gradual changes in other putative integrator functions

![Figure 9](http://jn.physiology.org/)

**FIG. 9.** Changes in optokinetic nystagmus (OKN) and other oculomotor behaviors associated with nph lesions. To compare, values have been normalized by dividing by the average initial value (~1.0 for lesion 0). Each point is the average of all values collected on the 14th through the final day of data collection after each lesion. A: sinusoidal (±10°) horizontal vestibulo-ocular reflex (0.5 Hz) gain (dots) and phase (circles), horizontal smooth pursuit gain (squares, 1.0 Hz), and VN peak velocity (diamonds) are constant over the series of injections compared with fixation (diamonds), which drops precipitously and immediately and never recovers. Standard deviations are the size of the symbols or smaller. B: representative OKN (90°/s) and VN (80°/s) metrics (see key) that declined gradually over the course of the lesions. Standard deviations ranged from symbol size to as much as the illustrated error bars. C: VN and OKN metrics that increased initially in association with nph damage and showed the highest variability; error bars = 1 SD.
suggest that the rostral nph is involved very little in those processes as detailed later in this DISCUSSION.

The latter conclusion differs markedly from the conclusion drawn from similar studies using excitatory neurotoxins in monkeys (Cannon and Robinson 1987) and cats (Cheron and Godaux 1987). The results of those two studies led the investigators to conclude that the nph is the single oculomotor neural integrator. However, there are several differences between those studies and the one reported here.

First, it should be pointed out that the monkeys in this study usually could not perform any oculomotor tasks immediately after an injection because the injections induced a large contralateral offset in the normal gaze and a resulting positional nystagmus whenever the animal looked away from that offset, “null,” position. These offsets were often so large that the animals’ eyes were pinned at the contralateral extreme of the oculomotor range except for a few ipsilateral quick phases. When this happened, it seemed pointless to test oculomotor behavior because the results were deemed uninterpretable. It was assumed that long-lasting deficits and permanent damage were more indicative of true nph function. In those few cases when the offset and nystagmus were not as severe, testing focused on the role of the nph in saccades so other behaviors were not examined routinely at the time of injection. In contrast, previous studies concentrated on the oculomotor deficits immediately after the injections.

Second, previous volumes and toxicity of neurotoxin were several times larger than the ones used in this study. Cheron and Godaux (1987) used kainate, which is 10 times more potent than ibotenate (Köhler 1983), and they used 1.0-μL volumes rather than the hundreds of nanoliters used in this study. Cannon and Robinson (1987) used both kainate and ibotenate, but their volumes were as much as fivefold larger than ours.

Third, the previous studies did not result in either permanent lesions of the nph or permanent deficits. The maximal extent of the permanent lesions due to ibotenic acid injections in our subjects was documented with the aid of glial fibrillar acidic protein immunohistochemistry, and the minimal extent was assessed in normal Nissl-stained material (Kaneko 1997). Both techniques verified the permanent and extensive damage to rostral nph in all three animals. The data in this paper confirm the gradual but minimal effects those lesions had on smooth pursuit, VOR, VN, and OKN.

Fourth, the two previous studies produced similar oculomotor deficits whether the injections were placed in the nph or in the neighboring medial vestibular nucleus (mVN), whereas in the present study, care was taken to avoid damage to the mVN. Thus it is possible that some of the differences observed are due to spread of the neurotoxin to the neighboring mVN at the time of the injection in those other studies.

On the basis of the differences between the earlier studies and the one described here, I suggest that the present results are more indicative of deficits unique to rostral prepositus lesion. Conclusions from previous investigations seem to be based on transitory effects of larger quantities of neurotoxin injection that may have spread to neighboring areas.

It is unlikely that the reason nph damage had minimal effects on some oculomotor behaviors was that the lesions missed the appropriate region of the nph. In every case, the marginal zone (the output region of the nph) (Langer et al. 1986) was affected severely, and in the most thoroughly documented case (monkey R), it probably was destroyed completely, at least on the right side. The GFAP material from monkey R suggests that the damage was much more extensive than could be estimated in the Nissl material from monkeys Z and M. In monkey Z, which received a large-volume injection that encroached on neighboring regions and resulted in frank mechanical damage to the many fiber tracks in the region, OKN and VN were affected more severely, suggesting that neighboring regions and/or fibers in the region contribute more directly to those behaviors, whereas nph does not. This conjecture is supported by the midline lesion study of Katz et al. (1991), who showed that section of the medullary commissure obliterated OKN and VN but left intact the ability to hold peripheral gaze, the VOR, and saccades.

Smooth pursuit

There was very little change in smooth pursuit, and what change was observed was gradual (Figs. 1 and 2). There are a number of possible explanations for the small changes observed at higher tracking frequencies. First, the phase shift and lower gain might suggest a loss of neural integrator function. This explanation would predict that the position input to the abducens is diminished, whereas the velocity input remains relatively unchanged and thus tracking should be more nearly in phase with target velocity. However, the data are not consistent with this prediction. The maximum ~20° phase shift for higher target velocities actually goes from a small lag, beyond 0° (i.e., in phase with target position), to a slight phase lead (~5° at 1.0 Hz; Fig. 1) so that the phase relative to position is improved (closer to 0). Second, the small changes may be due to adaptation and/or improved tracking. The gradual but steady decreases in gain argue against this interpretation, but it cannot be ruled out completely because, as mentioned, temporal accuracy (phase relative to position) is improved. Finally, the small changes may be due to damage to neighboring, nonintegrator neurons. Specifically, Cullen and colleagues (1993) have described eye-velocity neurons in and around the nph that they suggest are critically involved in horizontal smooth pursuit. The loss of such neurons would be expected to affect the gain of pursuit, as was observed. The data do not allow a definitive conclusion, but the small changes in gain suggest that the observed results are due to destruction of nearby pursuit neurons and not damage to the neural integrator per se. The decrease in the initial jump velocity of OKN is also consistent with the observed, modest decrease in pursuit gain (see Nystagmus later in this discussion).

The gradual course of changes in pursuit contrasts with the abrupt, permanent loss of ability to hold peripheral eye position after even a single lesion (Fig. 9A) (Kaneko 1997). Indeed, a preliminary study did not reveal any obvious change in pursuit (Lambert and Kaneko 1995). Only when the highest tracking frequencies are considered is the trend for slight shifts in gain and phase revealed. Previously, we have argued that the pursuit system may not require an integrator at all because smooth pursuit is not characterized by accurate eye position when it ends due to the long visual feedback delays (Fukushima and Kaneko 1995; Fukushima et al. 1992; Kaneko 1997). Because the velocity-to-position integrator is devastated so quickly and easily, whereas pursuit is affected only minimally, I conclude...
that the nph is associated with that portion of the integration process that maintains eye position after saccades in the dark and not with the putative pursuit integrator, if one exists.

**VOR**

The data presented in Figs. 3 and 4 show that the rostral nph is not a major component of the neural integrator for sinusoidal VOR. At the turn of the century, neural integration was postulated to account for the necessity to convert vestibular velocity-coded afferent information into eye-position commands. A number of studies (see Fukushima and Kaneko 1995 for review) have suggested that the integrative process includes several components that are dispersed anatomically. The data reported here suggest that the major substrate for vestibular neural integration resides outside of the nph, possibly in the vestibular nuclei, specifically the mvn for horizontal (yaw) vestibular stimuli. A possible role of the more caudal nph in the integrative process has been suggested by the extensive inter-nph and nph-mvn anatomic connections and by the effects of neuroactive agents injected into either region (e.g., Godaux et al. 1993). The present data cannot rule out such a contribution completely, but the extensive nph damage caused by our injections (Kaneko 1997) suggests that they may not be necessary. Certainly, the well-known direct vestibulo-ocular connections from various vestibular neurons (e.g., Scudder and Fuchs 1992) could relay the output of the integrative process from the mvn.

The curious changes in vertical sinusoidal VOR are not easily explained. The phase and gain changes occurred over the third to fifth lesions slightly after the point where the gaze-holding deficits were maximal (3rd to 4th lesions, Fig. 7 in Kaneko 1997). The increased gain remained high, and it is unlikely that it was a learned response as testing had been underway for more than a year when it began. The changes may reflect coupling with the adaptive components of the horizontal system (e.g., Figs. 6 and 9B) in response to the loss of nph function.

**Nystagmus**

The nystagmus results presented here are somewhat different from those of earlier reports on monkey (Cannon and Robinson 1987) and cat (Cheron and Godaux 1987). In both of those studies, injections greatly reduced eye-movement response to the stimulus. The response that remained was interpreted as a step in eye position due to crippling of the neural integrator. Because our animals always showed a persistent contralateral offset after an injection (Fig. 5 in Kaneko 1997) but were still capable of making OKN movements and never showed a position step, the step others observed may have been due to the offset and not to disabling of the neural integrator. Monkey Z, whose left nph was totally destroyed (see Fig. 2 in Kaneko 1997), had no OKN for ipsilateral drum rotation. Even so, this animal also had no step in horizontal eye position (Fig. 8C, bottom). Recall that the position trace is the average position without quick phases, which had been removed. It is also possible that the small unilateral injections in this study did not affect the nph sufficiently to reproduce the previous findings. This explanation seems unlikely because most of the nph was destroyed eventually and a position-step response to OKN stimulation never was observed.

The present results only partially support current models for the neural mechanisms of nystagmus. Because the velocity storage mechanism was first formalized as the basis for OKN and VN (Raphan et al. 1977), it has been considered to be a single process. Whereas the direct input, visual for OKN and VOR for VN, contributes to the initial component at stimulus onset and offset and may be separate, the storage process is supposed to be a single mechanism. The present data (Figs. 6–9) suggest a more complicated structure and further indicate which components may be linked. For example, although there were parallel transient increases in the time constants for charging and discharging for OKN and per- and postrotatory responses for VN (Fig. 9), these recovered to nearly normal or slightly lower than normal values. In contrast, the changes in steady-state OKN diminished progressively without recovery and did not show any transient increase (Fig. 9B), whereas the changes in peak plateau velocity were rather minimal (Fig. 9A). The differential progression clearly dissociated the charging/discharging process from the storage and may indicate differences in storage for optokinetic and vestibular velocity. Nonetheless, the parallel changes for OKN and VN charging/discharging strongly support the original concept of velocity storage that is accessed by both visual and vestibular input. Because changes in duration of the VN plateau paralleled the changes in time constant (Fig. 9), I suggest that the plateau of VN directly reflects the storage component and is not associated with the charging mechanism as measured directly by the time constants of charging and decay.

The data reported here are consistent with the interpretation that the initial nystagmic response is a result of the stimulus input. For VN, the initial component is supposed to be due to the VOR and, like the VOR (Figs. 3 and 4), the initial VN changed only modestly (Fig. 9B) over the course of the injections. For OKN, the initial response is supposed to be visual but the exact source(s) remain(s) uncertain (for discussion, see Fuchs and Mustari 1993). The changes in gain of the pursuit response (Fig. 1) were mimicked by the changes in the jump in OKN (Fig. 9) and, in both cases, the small decreases were most obvious at higher velocities. Thus it would seem that minor smooth pursuit deficits were due to damage to local pursuit neurons, and this also may explain the minor deficits in the initial jump in eye velocity during OKN.

The output of the velocity storage mechanism is thought to be relayed to oculomotor neurons via the common integrator (Raphan et al. 1977; Robinson 1977). In the current study, however, nph damage had no drastic effect on OKN or VN (Figs. 6–9), whereas it devastated gaze holding in an earlier study (Kaneko 1997). Thus the nystagmus position integrator either does not exist (it does not seem to be necessary) or is separate from the fixation integrator in the nph. This finding is consistent with the demonstration that midline section of the dorsal rostral medulla destroys velocity storage but not gaze holding (Katz et al. 1991). It may be that the output of velocity storage is distributed more widely than the position integrator alone, for destruction of the nph does seem to have some effect on storage. Steady-state velocity decreased and the initial drop in OKAN increased, whereas the jump in OKN decreased (Fig. 9). In addition, the OKAN (discharging) time constant decreased, whereas the OKN time constant was relatively unaffected (Fig. 9). These results suggest that eye velocity may be derived partially from the nph environs, possibly from the...
same eye-velocity neurons that account for the minor pursuit deficits, but that the major effects of nph lesions are to make the velocity storage integrator more “leaky.” This leakiness also was seen in the decline of the VN time constants, but the peak plateau velocity for VN did not decline like OKN steady-state velocity (Fig. 9).

**Conclusions**

Previous studies in this laboratory led to the conclusion that the nph in general, and the marginal zone in particular, is not necessary for production of normal saccades. They also showed that the position feedback integrator for saccades is not the same as the integrator for stable gaze (Kaneko 1997). The present results show that the nph also is not necessary for normal smooth pursuit or the sinusoidal VOR in the dark. Therefore, I conclude that there is no common integrator for horizontal eye movements. Nonetheless, this conclusion remains controversial. For example, one recent study reached the opposite conclusion because nph recordings showed that position sensitivity of nph neurons was identical for fixation and VOR (Godaux and Cheron 1996). However, the statistical test used in that study may have been inappropriate because the low sampling rate and smoothing of the eye-movement signal altered the error metric. In contrast, another recent study, like the present one, concluded that the neural integrator is distributed; this conclusion was based on muscimol inactivation of the vertical integrator in the interstitial nucleus of Cajal (Helmchen et al. 1998). One of Robinson’s strongest arguments in favor of a common integrator (Robinson 1968, 1989) was that the final eye position dictated by all the various oculomotor subsystems must be coordinated consistently. As the oculomotor system seems to use multiple and/or distributed neural integrators, study of the neural integrators now should focus on how the components are coordinated to provide a uniform position signal to the motoneurons and on which systems actually rely on a velocity-to-position neural integrator.

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