Oscillatory Eye Movements Resembling Pendular Nystagmus in Normal Juvenile Macaques

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Purpose. Juvenile monkeys being trained on smooth-pursuit tasks exhibit ocular oscillations resembling pendular nystagmus. The purpose of this study was to analyze these oscillations, the effects of gabapentin on them, and responses of cerebellar floccular neurons to understand possible neuronal mechanisms.

Methods. Four monkeys were trained for horizontal and vertical smooth pursuit; in two, saccades were also tested. Frequency, peak-to-peak eye velocity, and amplitude of the ocular oscillations were measured. In one monkey, the effect of gabapentin on the oscillations was measured, and oscillation-related neuronal discharge was recorded in the cerebellar floccular region.

Results. Ocular oscillations, with features of pendular nystagmus, appeared early during training of both horizontal and vertical pursuit in all four monkeys. Although these oscillations were observed both in the direction of pursuit and orthogonally, the velocity and amplitude of oscillation were larger in the direction of pursuit, implicating pursuit mechanisms in their generation. Corrective saccades were often superimposed on the oscillations during pursuit and fixation. Gabapentin suppressed oscillations in the monkey tested. Recordings in the floccular region revealed a subset of neurons discharged during both the oscillations and corrective saccades. Many of them exhibited burst-tonic discharge during visually guided saccades, similar to discharge of brain stem burst-tonic neurons, suggesting contributions of the neural integrator to the oscillations.

Conclusions. The developmentally transient ocular oscillations occurring in monkeys during pursuit training has properties resembling pendular nystagmus. Both smooth pursuit and a neural integrator may contribute to these ocular oscillations. Analysis using an efference-copy pursuit model supports the interpretation herein. (*Invest Ophthalmol Vis Sci.* 2011;52:3458–3467) DOI:10.1167/iovs.10-5903

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Nystagmus is defined as repetitive, to-and-fro involuntary eye movements that are initiated by slow drifts.[1] It is traditionally classified as jerk and pendular types by its waveform. Pendular types of nystagmus encompass infantile forms and acquired pendular nystagmus resulting from neurologic disease (Table 1). In monkeys a high-frequency form of pendular nystagmus has been induced by early-onset visual deprivation; jerk ("latent") nystagmus was also present.[2] The neural mechanisms of infantile pendular nystagmus are not well understood, but Jacobs and Dell'Osso[3] hypothesize that it may be due to a loss of damping of velocity oscillation inherent in the pursuit system.

Acquired pendular nystagmus occurs with disorders of myelin, such as multiple sclerosis (MS) and in the syndrome of oculopalatal tremor (OPT).[1] One hypothesis proposed for the pathogenesis of pendular nystagmus with MS is that it represents instability in the neural integrator that, in health, guarantees steady gaze. Evidence to support this hypothesis comes from the common observation that a fast eye movement (saccade) will often "reset" the oscillations or even transiently stop the pendular nystagmus.[4] However, no animal model for this form of pendular nystagmus exists, and as a result, responsible neuronal mechanisms are not well understood.

The syndrome of OPT usually follows a brain stem stroke, which interrupts projections from the deep cerebellar nuclei to the inferior olivary nucleus. A recent hypothesis to account for OPT is that it represents abnormal electrotonic coupling of neurons in the inferior olivary nucleus, leading to synchronized discharge at approximately 2 Hz, which causes maladaptive learning by the cerebellum.[5] The ocular oscillations of OPT are less regular than the pendular oscillations of MS and have the appearance of the sum of several oscillations, each at a different frequency.

One important difference between congenital and acquired forms of pendular nystagmus is that the congenital form often shows "foveation periods," during which the fovea of the retina is pointed at some feature of interest and the eyes are briefly still.[1,6–8] Foveation periods are not a feature of acquired pendular nystagmus (Table 1). A second important difference between congenital and acquired forms of pendular nystagmus is that the former is mainly horizontal in direction,[1,6–9] whereas the latter often has vertical or torsional components, either of which is sometimes predominant.[10–15] A third difference concerns the conjugacy of pendular nystagmus, which is

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TABLE 1. Clinical Features of Congenital Pendular Nystagmus, Acquired Pendular Nystagmus, and Oscillatory Eye Movements in the Present Study

	Congenital Pendular Nystagmus*	Acquired Pendular Nystagmus*	Oscillatory Eye Movements in the Present Study
Direction	Usually horizontal	May have horizontal, vertical, and torsional components	Horizontal and vertical (torsional component not recorded)
Frequency, Hz	0.5–8	1–8†	0.9–7.8
Amplitude, deg	0.3–30	1–8	0.1–4.7
Monocular/binocular	Usually binocular	Monocular/binocular	Binocular
Conjugate/disconjugate	Usually conjugate	Conjugate/disconjugate	Conjugate
Foveation period	Often present	Absent	Absent

* Clinical features of congenital and acquired pendular nystagmus were obtained from References [1,5–16].
† Acquired pendular nystagmus associated with OPT is typically 2–4 Hz but is often the sum of several frequencies.

the rule for congenital forms[6–8] but often is not observed in acquired forms,[10–15] due to coexistent brain stem lesions, such as internuclear ophthalmoplegia. Acquired pendular nystagmus due to MS is often suppressed by the drug gabapentin,[15,16] which acts at calcium channels[17]; some individuals with the congenital form of pendular nystagmus also benefit from this drug.[9]

Recently our laboratory has reported that, during early pursuit training, juvenile monkeys exhibited transient oscillatory eye movements that looked very similar to pendular nystagmus.[18] We analyzed the characteristics of the oscillatory eye movements and tested the effects of administering gabapentin on the oscillations. To investigate possible neuronal mechanisms of the oscillations, we recorded neuronal activity correlated with the oscillations in the cerebellar flocculus and also performed model analysis.

METHODS

Four juvenile monkeys (J, S, Y, N; *Macaca fuscata*, 3.0–3.6 kg) were used. Three of these monkeys (4 years old) were the same monkeys that were used in recent studies from our laboratory.[18] One monkey (N) was 5.5 years old. All procedures were performed in strict compliance with the guidelines for the Care and Use of Animals of the National Institutes of Health and with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Our protocols were approved by the Animal Care and Use Committee of Hokkaido University School of Medicine. Each monkey was sedated with ketamine hydrochloride (5 mg/kg, IM) and then anesthetized with pentobarbital sodium (25 mg/kg, IP). Under aseptic conditions, head holders were installed to restrain the head firmly in the primate chair during recording sessions. A scleral search coil was implanted on each eye to record the vertical and horizontal components of eye movement for both eyes.[19] Analgesics and antibiotics were administered postsurgically. In one monkey (S), two screws were implanted in the skull over the frontal and occipital cortex to record the electroencephalogram (EEG). In another (monkey N), a recording cylinder was implanted to allow single-neuron recording in the cerebellar flocculus region.

Each monkey was seated in a primate chair in darkness with the head restrained in the stereotaxic plane, facing a 22-inch computer display placed 65 cm distant from the eyes. A red spot of 0.5° angular size was used as the target. During the initial training, which lasted for 3 to 5 days and ~30 minutes per day, our monkeys were trained only in horizontal pursuit of sinusoidal target motion at 0.2 Hz (±10°, peak target velocity 12.6°/second). In all behavioral experiments, the same sinusoidal pursuit stimulus was used. Reward circuits compared target position signals with the monkeys' eye position signals. If the monkeys' gaze was within the error window of ±5° for 0.5 seconds, a drop of apple juice was automatically delivered to the monkeys. We set the reward window wider compared to the previous studies (e.g., Ref. 20, ±1° for 0.5–1 second) to study pursuit eye movements from the very beginning of training.[18] This was to allow the inexperienced monkeys to increase proficiency at tracking. Once the gain of horizontal pursuit reached 0.7, we started training the monkeys at vertical pursuit for ~2–3 weeks at 0.2 Hz (±10°), ~30 minutes per day, 5 days per week. During these training periods, horizontal pursuit was only briefly tested, typically for 5 minutes, before testing vertical pursuit. Eye position signals were calibrated by requiring the animals to fixate a stationary target of a known angle before each recording session.

In two monkeys (J, N), saccadic eye movements were also tested. For this, the target was positioned at 1°, 2°, or 5° to the left or right of the central fixation point. This was to compare peak eye velocities during small amplitude saccades to those of the oscillatory eye movements.

In one monkey (N), the effect of gabapentin, which was reported to be effective in the treatment of acquired pendular nystagmus,[9,15,16] was studied. After the clinical procedure,[16] gabapentin was given at a starting dose of 50 mg per day for 2 days, then 100 mg per day for 2 days, then 150 mg per day for 2 days, and finally 200 mg per day for 7 days. After the end of the 13-day treatment period, the dose of gabapentin was tapered and stopped within 6 days. Eye movements were recorded during horizontal pursuit at 0.2 Hz (±10°) for 760 seconds for each day tested. The baseline data were collected for 2 days: on day 5 after the beginning of initial training and the day just before the treatment started which was 6 month after the initial training. The data recorded during gabapentin treatment were obtained on days 11, 14, and 15 after the treatment started. The data obtained after treatment were from days 7 and 8 after the drug was stopped.

In monkey N, we also made extracellular neuronal recordings in the cerebellar floccular region as reported previously[21,22] while searching for neurons whose discharge was modulated in relation to corrective saccades (see Results). The floccular region, well recognized as necessary for generating accurate oculomotor pursuit,[1] was chosen for investigating the neurology of the oscillatory eye movements (see Ref. 1 for a review). Once modulated neurons were encountered, their activity was recorded during pursuit at 0.2–0.3 Hz and during saccades and fixation.

During training, an infrared camera monitored the faces, as a means of monitoring alertness, of all monkeys tested. In one monkey (S), EEG activity was also carefully monitored as an indicator of drowsiness (e.g., Ref. 23). In all monkeys tested, we collected data only when the monkeys performed the task. Once a monkey lost motivation to perform the smooth-pursuit and/or visually induced saccade task (detected by monitoring the eye movement recordings and the infrared camera), we discontinued recordings for that day.

Data Analysis

Eye position signals were digitized at 500 Hz using a 16-bit A/D board (National Instruments, Austin, TX) on a desktop computer (Macintosh Quadra; Apple, Cupertino, CA). Eye coil position signals from both eyes were differentiated by analog circuits (DC, 100 Hz, −12 dB/octave) to obtain velocity. Poor pursuit performance (monkeys occasionally did not pursue the target) as judged from the eye position

traces was not considered, and only those traces in which monkeys pursued the target were accepted for analysis. As illustrated in Figure 1 (indicated by x), we measured the frequency of the oscillatory eye movements by manually positioning a vertical cursor on the computer monitor at the peak of a single oscillatory eye velocity cycle. Similarly, we measured peak-to-peak eye velocity of the oscillatory eye movements by manually positioning a horizontal cursor on the peak of a single oscillatory eye velocity cycle (Fig. 1, indicated by *y*). Peak-to-peak amplitude of oscillation was also measured by manually positioning a horizontal cursor on the peak of each oscillatory eye position trace. For this, any eye position change due to pursuit was canceled out in the eye position trace (Fig. 1, p-p amplitude). Horizontal and vertical oscillatory eye velocity was analyzed separately; we measured parameters of oscillation only for those oscillatory cycles that were clearly visible in eye position records (see Fig. 1) to avoid misidentifying artifacts generated during the electrical differentiation process.

In monkeys J, S, and Y, we reanalyzed the data obtained in the previous study.[18] Data from 6, 3, and 12 days (30 minutes for each day) were included for monkey J, S, and Y, respectively. For monkey N, data for 6 days were included.

To evaluate the effects of gabapentin, the total fractional percentage (seconds) of oscillatory eye movements during horizontal pursuit within a 760-second recording period were compared before and during gabapentin treatment. We did not compare oscillations after treatment; since sometimes gabapentin induces lasting changes in acquired pendular nystagmus in human subjects,[16] we suspected similar effects in our data. Pursuit eye velocity gain was calculated as described previously[18] by aligning greater than 100 de-saccaded eye velocity traces with stimulus velocity for calculation of mean and SD to evaluate the effects of gabapentin.

The effects of gabapentin were also evaluated on each cycle of sinusoidal pursuit by plotting eye velocity gains against mean square error before and during gabapentin treatment. The mean square error represents the error about the best-fit relationship between eye and target velocity derived by regression analysis. All cycles including those with oscillation where the monkey tracked the target reasonably well judging from eye position trace were plotted.

To analyze neuronal discharge during oscillatory eye movements and/or corrective saccades (see Results), all traces were aligned with peak eye velocity. For this, eye velocity was obtained by differentiating eye position traces in the computer using a 6 ms time window.

Recording locations of responsive neurons in the floccular region were confirmed histologically as described earlier.[21,22]

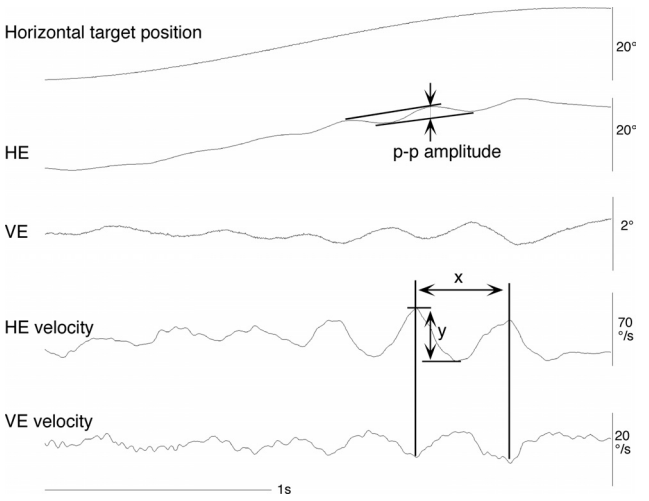

Figure 1. Oscillatory eye movements during smooth-pursuit training. HE, horizontal eye position; VE, vertical eye position; HE velocity, horizontal eye velocity; VE velocity, vertical eye velocity.

Results

Characteristics of Oscillatory Eye Movements during Pursuit

During 2 to 3 weeks of pursuit training in four juvenile monkeys, ocular oscillations resembling pendular nystagmus frequently appeared in days 6 to 14 in monkey J, in days 5 to 12 in monkey S, in days 2 to 17 in monkey Y, and in all days in monkey N. The oscillatory eye movements appeared during both horizontal pursuit and vertical pursuit. During these periods, horizontal pursuit gains were ~0.7–0.8 in all four monkeys, but vertical pursuit gains were always less, and, particularly, upward pursuit gains were ~0.4–0.5.[18] We observed carefully the monkeys' eye movements using an infrared camera. During these oscillatory eye movements, all monkeys tested performed the task with their eyes wide open, suggesting that the oscillatory eye movements were not due to a loss of alertness or due to incessant blinking. This was also confirmed by carefully observing the EEG in one monkey; a low-voltage, fast EEG pattern was always observed during these oscillatory eye movements.[23] By turning off the target briefly (e.g., for 5 seconds), these oscillatory eye movements disappeared, but they then reappeared in 1 to 2 minutes if the target was turned on and pursuit training resumed. After 3 weeks of training, these oscillatory eye movements rarely appeared in three of the four monkeys tested (J, S, Y). In monkey N, however, oscillatory eye movements continued to appear for 6 months.

Oscillatory eye movements recorded from both eyes were virtually identical and appeared during both horizontal pursuit and vertical pursuit in all four monkeys tested, and they were conjugate (data not shown). Representative records of oscillatory eye movements from one eye are illustrated in Figure 1. When the monkey pursued a spot moving horizontally, both horizontal and vertical eye position traces exhibited oscillatory movements (Fig. 1, HE, VE). The oscillation is clear in eye velocity traces with nearly identical phase relationship (Fig. 1, HE velocity, VE velocity; note higher gains of vertical eye position and velocity calibration).

Mean (±SD) frequencies (Hz) of oscillatory eye movements for four monkeys were 3.03 (±0.72) for horizontal oscillation during horizontal pursuit (HH), 3.23 (±0.79) for vertical oscillation during horizontal pursuit (VH), 3.34 (±1.18) for horizontal oscillation during vertical pursuit (HV), and 3.79 (±0.99) for vertical oscillation during vertical pursuit (VV). Mean (±SD) amplitudes of the oscillatory eye movements were 1.39 (±0.77) for HH, 0.55 (±0.31) for VH, 0.58 (±0.36) for HV, and 1.06 (±0.67) for VV. Mean (±SD) peak-to-peak velocities of the oscillatory eye movements were 30.71 (±10.75) for HH, 11.03 (±4.95) for VH, 11.30 (±4.95) for HV, and 27.97 (±11.72) for VV. Three-way ANOVA test with three factors (monkey, target direction and oscillation direction) showed significant dependence of oscillation frequency on monkey factor ($P < 0.01$), while amplitude and velocity of oscillations did not depend on monkey factor ($P > 0.5$ and $P > 0.3$, respectively).

Figure 2 summarizes the frequency (A), peak-to-peak amplitude (B), and peak-to-peak velocity (C) of oscillatory eye movements. Data pooled for four monkeys are illustrated as box plots for four types of oscillations (HH, VH, HV, VV). Each plot shows median (red line) and the 95% confidence interval around median (notches inside blue box). Any pair of overlapping notches indicates the absence of significant differences between oscillation types. We used the Kruskal-Wallis test to reveal significant difference among the four oscillation types. For pairwise comparisons, we used the Wilcoxon rank sum test. Four group Kruskal-Wallis tests showed significant

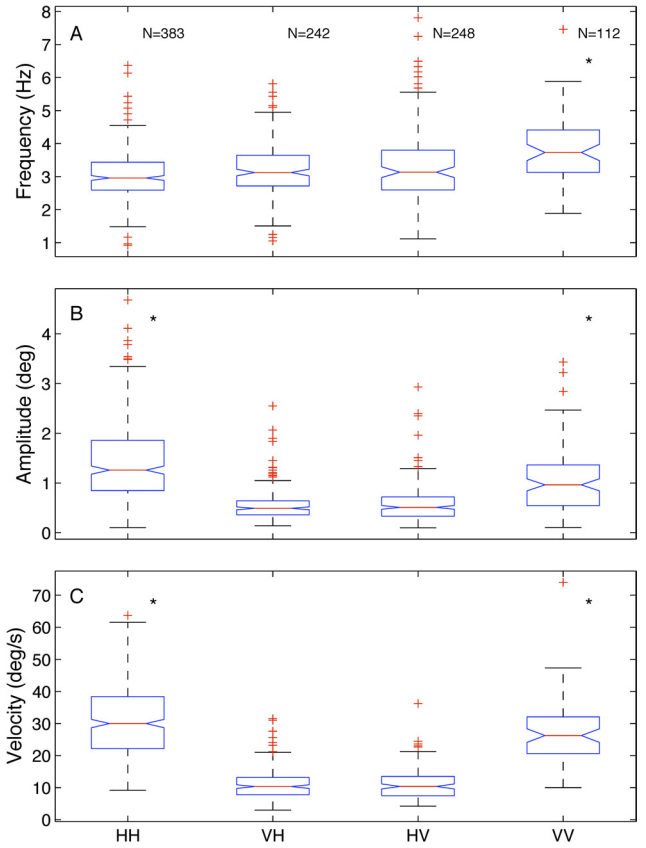

FIGURE 2. Box plots of frequency (**A**), peak-to-peak amplitude (**B**), and peak-to-peak velocity (**C**) of oscillations in four conditions: horizontal oscillations during horizontal pursuit (HH), vertical oscillations during horizontal pursuit (VH), horizontal oscillations during vertical pursuit (HV), and vertical oscillations during vertical pursuit (VV). In each box plot: *red line*: median value; *blue box*: 25% and 75% quartiles; *whisker*: range of data (the most extreme values within 1.5 times the interquartile range from the ends of the box); *red crosses*: outliers; *notches*: confidence interval around median; *N*: number of trials pooled for three monkeys; *asterisks*: significant differences ($P < 0.05$). Data for 6 days and 3 days (30 minutes for each day) were included for monkeys J and S, respectively. For monkey N, data for 6 days were included.

changes in each of three measured parameters for the four types of oscillations. Figure 2A shows that the median frequency (3.73 Hz [95% confidence interval (CI), 3.48–3.98]) of VV was slightly but significantly higher than the median frequency of other types of oscillations (HH, VH, HV, pooled median 3.08 Hz [95% CI, 3.02–3.13]). Because frequency of oscillations depended on monkey factor, we performed similar statistical tests for each monkey. The VV frequency was highest in three monkeys tested (the fourth monkey S was tested only in two conditions and was not included). The VV frequency was significantly higher than HH frequency in all tested monkeys; also in each monkey it significantly exceeded VH and/or HV frequency.

In contrast to the frequency, peak-to-peak amplitude and velocity of oscillations demonstrated consistent differences confirmed in three monkeys. As shown in Figure 2B, peak-to-peak amplitudes of oscillations in the pursuit direction (1.20°, pooled median for HH and VV [1.13–1.27, 95% CI]) were larger than those in the direction orthogonal to pursuit (0.50°, pooled median for VH and VV [0.48–0.52, 95% CI]). Figure 2C shows that peak-to-peak oscillation velocities in the pursuit direction (28.87°/seconds, pooled median for HH and VV [95% CI, 27.72–30.02]) were approximately three times larger than those in the orthogonal direction (10.35 °/seconds, pooled median for VH and HV [95% CI, 9.89–10.82]).

To examine whether frequencies and peak-to-peak velocities of oscillation changed with time during ongoing pursuit training, we compared their distribution by dividing each day's pursuit experience into three periods. Both the mean frequency and the peak-to-peak velocity of oscillatory eye movements did not show any clear change across the three periods within each day (data not shown). We also examined whether the distribution of these variables changed with the progress of pursuit training by comparing them on days 2–3, 4–8, 9–12, and 13–17. Both the mean frequency and the peak-to-peak velocity of oscillatory eye movements did not show any patterns of change across those four periods.

Effects of Gabapentin on Oscillatory Eye Movements

We evaluated the effect of gabapentin on oscillatory eye movements in monkey N. The total fractional percentage (seconds) of oscillatory eye movements recorded within a 760-second epoch of 0.2 Hz horizontal pursuit (±10°) (see Methods) is summarized in Figure 3A. Mean (±SE) duration of oscillatory eye movements was 97.3 ± 50.9 seconds at baseline before gabapentin treatment. During the treatment, the mean (±SE) duration was greatly decreased to 4.8 ± 0.7 seconds (Welch's *t*-test, $P < 0.05$). After drug administration ceased, the mean

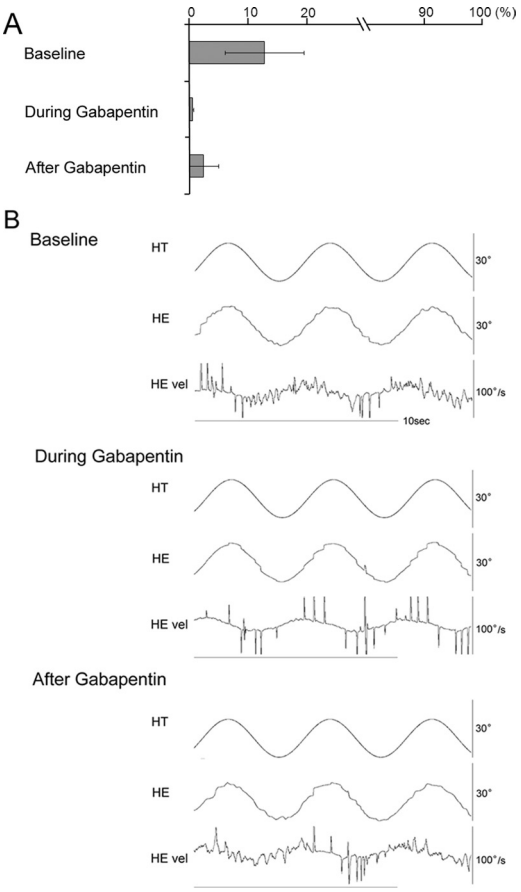

FIGURE 3. Effect of gabapentin. (**A**) Histograms show cumulative time extent of oscillatory eye movements recorded within 760-second epochs of pursuit training recorded before ("baseline"), during the time of peak effect of and after recovery from gabapentin infusion. (**B**) Representative records from each of the conditions, as labeled at left. Error bars (**A**) indicate SE.

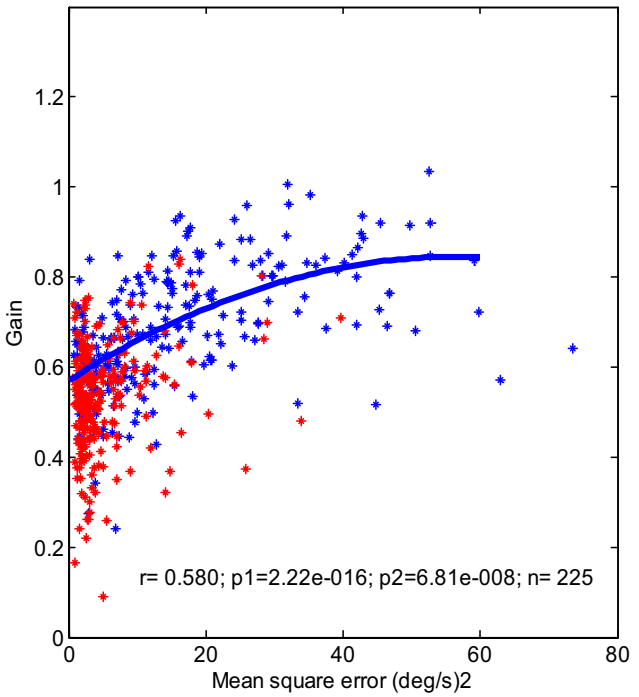

FIGURE 4. Effects of gabapentin on gain and error during sinusoidal pursuit in the baseline condition (*blue*) and during gabapentin treatment (*red*). Gain (eye velocity/target velocity) was derived by regression analysis; it is plotted against mean square error, which represents the error about the best-fit relationship between eye and target velocity. Each symbol represents gain and mean square error for one response cycle. A polynomial regression of gain versus mean square error for the baseline condition gave a significant fit to the data with overall correlation (*r*) and *P*. p1, linear; p2, quadratic.

(±SE) duration was 19.0 ± 19.0 seconds. Representative eye movement recordings from these conditions are shown in Figure 3B.

Mean pursuit eye velocity gains (±SD) during these three periods during cycles where oscillation did not appear were analyzed (see Data Analysis) and were 0.68 ± 0.07, 0.60 ± 0.04, and 0.64 ± 0.02, respectively. Mean eye velocity gain during gabapentin treatment was not significantly different from that before treatment (Welch's *t*-test, $P > 0.05$).

To illustrate the effects of gabapentin on each cycle of sinusoidal pursuit, Figure 4 plots eye velocity gains against mean square error during the baseline condition (blue) and during gabapentin treatment (red). All cycles including those with oscillation were plotted (see Methods). Cycles with larger mean square error were associated with greater oscillation. There was a significant correlation between gain and mean square error in the baseline condition, reflecting the fact that increases in gain were associated with greater oscillation. Baseline and gabapentin responses appeared to fall within the same distribution of gain versus mean square error, but mean gain was slightly higher in the baseline condition. Compared to the distribution during the baseline, with its larger range of mean square error (blue), gabapentin clearly reduced the error, and mean gain was also therefore slightly decreased (red).

Comparison with Saccades

To test the possibility that the oscillatory eye movements during pursuit were small saccades that occurred during pursuit training, we compared the peak velocity-amplitude relationship of visually induced saccades to that of the oscillatory eye movements (see Methods). The results are summarized in Figure 5 for horizontal and vertical components as A and B, respectively. For significant correlations, regression lines are shown with slopes and correlation coefficients. For the horizontal peak eye velocity versus amplitude relationship depicted in Figure 5A, horizontal oscillations during vertical pursuit had significant correlation (blue, $r = 0.66$, $P < 0.01$), but horizontal oscillation during horizontal pursuit did not have (red, $r = 0.1$, $P > 0.1$). For the vertical peak eye velocity versus amplitude relationship depicted in Figure 5B, vertical oscillations had significant correlation during both horizontal and vertical pursuit (red and blue diamonds, $r = 0.84$ and 0.52, respectively). However, none of these correlations reached the velocity amplitude ratios (i.e., slopes, 4.86–11.33 deg/seconds/deg) comparable to those of normal saccades (54.23–58.84 deg/seconds/deg). Thus, the two kinds of eye movements exhibited clearly different velocity-amplitude relationships.

Monkey *n* exhibited oscillatory eye movements during fixation after visually induced saccades, as illustrated in Figure 6A, where a visually induced saccade (*) was followed by corrective saccades (open arrowheads) and then by oscillatory eye movements (filled arrows). The peak eye velocity-amplitude relationship for corrective saccades (circles, Fig. 6B) and that

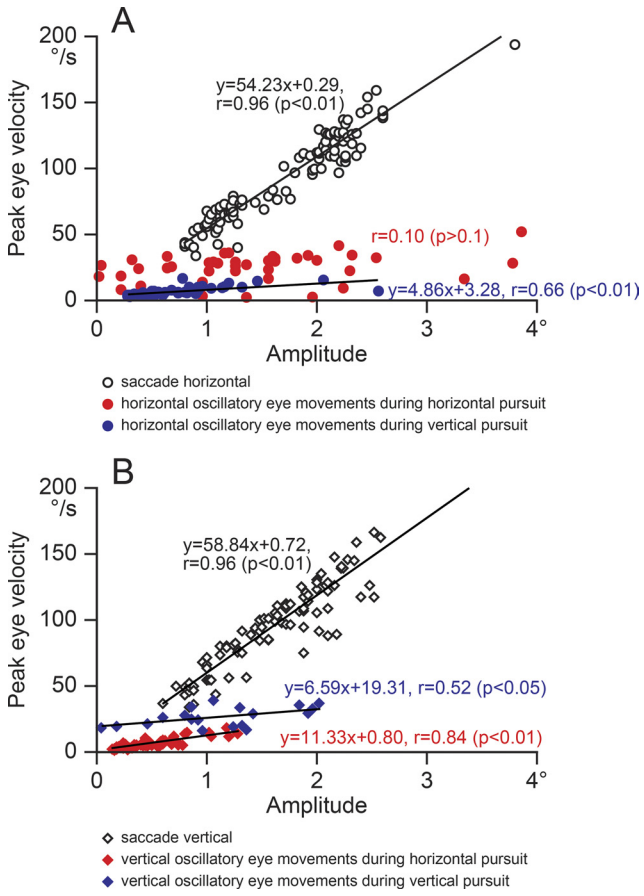

FIGURE 5. Comparison of eye movement peak velocity versus amplitude relations across saccadic and pursuit versus oscillatory eye movement behaviors. Peak eye velocity is plotted against eye movement amplitudes of horizontal components (**A**) and vertical components (**B**). *Open circles*: horizontal saccades; *red dots*: horizontal oscillatory eye movements during horizontal pursuit; *blue dots*: horizontal oscillatory eye movements during vertical pursuit; *open diamonds*: vertical saccades; *red diamonds*: vertical oscillatory eye movements during horizontal pursuit; *blue diamonds*: vertical oscillatory eye movements during vertical pursuit. Linear regression and correlation coefficients were calculated for significant correlation as indicated.

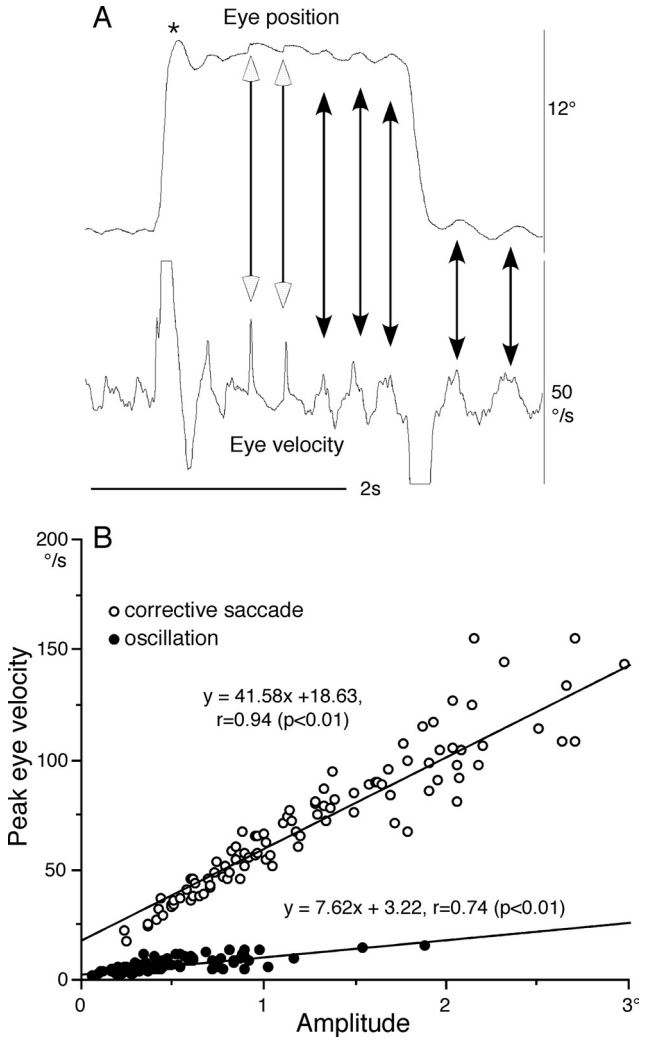

FIGURE 6. Oscillation during fixation and peak velocity amplitude relationship. (**A**) Corrective saccades (*open arrowheads*) and oscillatory eye movements during fixation after visually induced saccades (*filled arrows*). (**B**) Peak eye velocity against amplitude for corrective saccades (*open circles*) and oscillatory eye movements (*dots*). Linear regression and correlation coefficients were calculated for each as indicated.

for oscillatory eye movements (dots, Fig. 6B) are plotted in Figure 6B with their associated regression lines and correlation coefficients. The velocity amplitude ratio (i.e., slope) was clearly different between the two (41.58 vs. 7.62 deg/seconds/deg), and the relation of each to the other is similar to that of the slopes shown in Figure 5 for visually induced saccades. These results suggest different neural origins for the oscillations versus the small saccades.

However, careful observation of eye movement traces (Fig. 6A) indicated that corrective saccades often interchanged with oscillatory eye movements, suggesting an involvement of a common factor in the generation of the two kinds of eye movements. Although smaller saccades such as those in Figure 6A did not substantially change the frequency of oscillations, the first, large saccade (*) did appear to induce a phase shift in the oscillations when compared with the oscillations that preceded it. We, therefore, in an attempt to understand better these oscillations, recorded single-neuron activity related to corrective saccades and/or oscillatory eye movements in the cerebellar floccular region, an area known to be necessary for generating pursuit eye movements.[1,24]

TABLE 2. Classification into 3 Groups of Non-Purkinje Cell Neurons in the Floccular Region whose Activity Was Modulated during Corrective Saccades to a Slowly Moving Target

Group	Oscillation	Smooth Pursuit	*n*
1	Yes	No	8
2	Yes	Yes	21
3	No	Yes/no	6

Oscillation-Related Neuronal Discharge in the Cerebellar Floccular Region

We recorded a total of 65 neurons in the floccular region during pursuit and/or oscillatory eye movements. Of these, the activity of 35 neurons was modulated during corrective saccades. We classified these corrective saccade-responding neurons as belonging to one of three groups on the basis of whether they discharged in relation to oscillatory eye movements and smooth pursuit. Table 2 summarizes this classification.

The first group exhibited discharge modulation during oscillation but not during smooth pursuit (Table 2, group 1, $n = 8$). Discharge of a representative first-group neuron recorded in the right flocculus is illustrated in Figures 7 and 8. It discharged during leftward corrective saccades and also discharged during leftward oscillatory eye movements, but its discharge was not related to smooth pursuit (Fig. 7). When the discharge of this neuron was aligned with peak eye velocity recorded during leftward corrective saccades, it discharged nearly simultaneously with corrective saccade onset (Fig. 8A). It also discharged in association with visually induced leftward saccades (Fig. 8C). During the oscillatory eye movements recorded while the monkey was pursuing, its discharge increased only in relation to leftward oscillatory eye velocity (Fig. 8B), where peak discharge was found to phase-lead peak oscillatory eye velocity by, typically, 20 ms.

The second group of floccular neurons (Table 2, group 2) exhibited discharge modulation during oscillatory eye movements and also during smooth pursuit ($n = 21$). The records of a representative neuron recorded in the right flocculus are illustrated in Figure 9. This neuron discharged clearly during rightward pursuit and also discharged during rightward oscillation and corrective saccades (Fig. 9A). When discharge of this neuron was aligned with peak eye velocity during rightward corrective saccades (Fig. 9C) and oscillatory eye movements

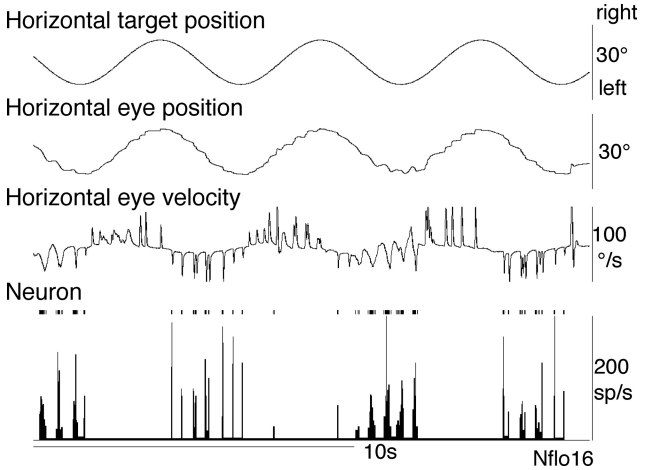

FIGURE 7. Discharge during pursuit of a representative group I non-Purkinje oscillation neuron in the cerebellar floccular region.

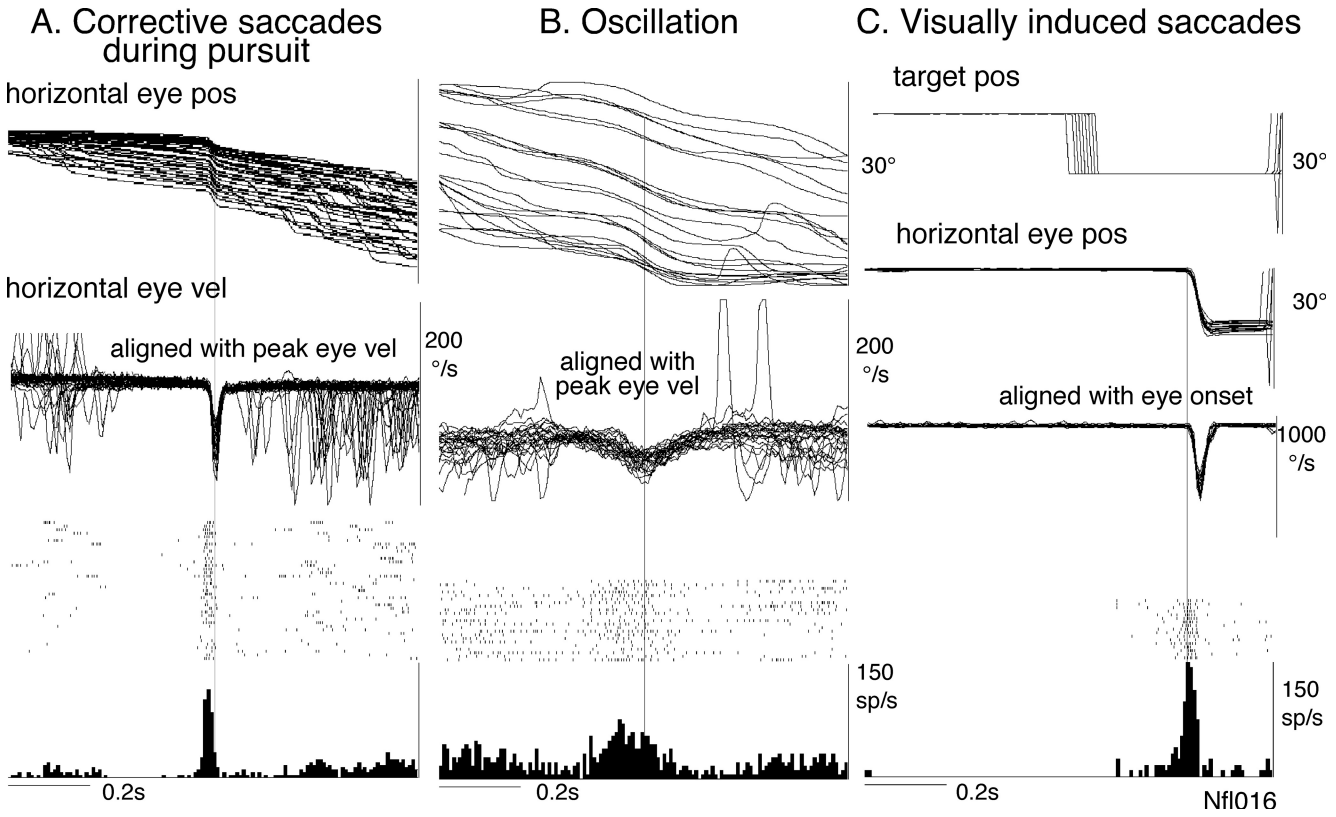

FIGURE 8. Quantitative analysis of discharge characteristics of a group I neuron during corrective saccades and oscillation. (This neuron is the same neuron shown in Figure 7.) Temporal characteristics of neuronal discharge and eye movements are shown by superimposing multiple records of corrective saccades (**A**), oscillatory eye movements (**B**), and visually induced saccades (**C**). pos, position; vel, velocity.

(Fig. 9D), the peak burst discharge aligned well with the onset of both types of eye movements. Moreover, during visually induced saccades, it showed eye position related discharge during rightward saccades, thus exhibiting a burst-tonic type discharge in relation to saccades (Fig. 9B).

We recorded bilaterally from the floccular region. We found both group I and II neurons from both floccular regions. None of the group 1 and 2 neurons (Table 2, $n = 29$) were Purkinje cells, as identified by absence of associated complex spike activity and size; those neurons were recorded in the molecular layer, typically in close proximity to Purkinje cells (see Discussion).

The third group of neurons (Table 2, group 3) exhibited discharge during corrective saccades, but their activity was not modulated during oscillatory eye movements ($n = 6$, not shown). Some of them ($n = 3$) were Purkinje cells, as identified by their low-frequency complex spike activity associated with a pause in the recorded simple spike activity.

The remaining 30 neurons (of the 65) were pursuit-related Purkinje cells, but none of the 30 exhibited modulation related to oscillatory eye movements or to corrective saccades.

The subsequently reconstructed recording locations of responsive neurons were found in both the flocculus and ventral paraflocculus.[21,22]

DISCUSSION

Ocular Oscillations Resembling Pendular Nystagmus in Normal Juvenile Monkeys

We report that, during early pursuit training of a target spot moving either horizontally or vertically, our juvenile monkeys often exhibited oscillatory eye movements both in vertical and horizontal directions. Ocular oscillations resembling pendular nystagmus in the present study were not observed during sinusoidal pursuit in normal adult monkeys or in well-trained juvenile monkeys (e.g., Refs. 20, 25).

Comparison of Oscillatory Eye Movements in Congenital and Acquired Pendular Nystagmus and Normal Monkeys

Table 1 compares the characteristics of oscillatory eye movements in congenital and acquired pendular nystagmus (see Introduction) and in the normal monkeys of the present study. In the present study, oscillation was observed in both horizontal and vertical directions in all four monkeys tested (e.g., Fig. 1), although oscillations in the pursuit direction predominated (Fig. 2). We did not record torsional components in the present study. Frequencies of oscillation were similar across all three types of oscillatory eye movements and ranged from <1 to 8 Hz (Table 1). Amplitudes of oscillation of the present results were similar to those of both acquired and congenital pendular nystagmus, although patients with congenital nystagmus often have large amplitude oscillation. No "foveation periods" were observed in monkeys and acquired pendular nystagmus (Table 1). Finally, oscillations of the present study were observed binocularly and conjugately (Table 1), similar to the oscillations of congenital pendular nystagmus, which is consistent with acquired forms that lack coexistent brain stem lesions such as internuclear ophthalmoplegia. Thus, the oscillatory eye movements in the present study have features that are observed in both acquired and congenital pendular nystagmus. The oscillations observed in our monkeys' were more regular than those reported in OPT[5] and are more similar to those reported with MS.[4] The ab-

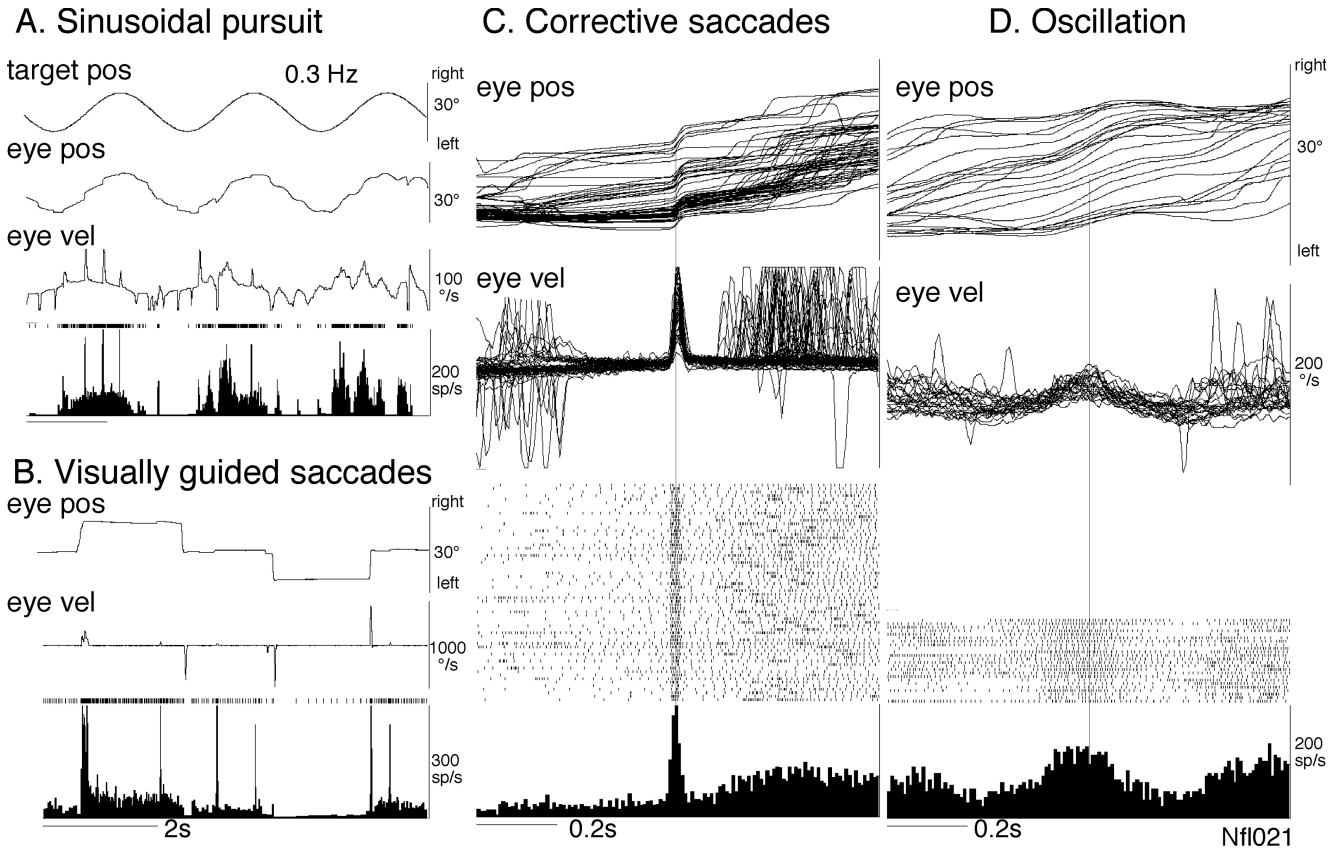

FIGURE 9. Quantitative analysis of discharge characteristics of a representative group II non-Purkinje oscillation neuron in the cerebellar floccular region. Shown are discharge during sinusoidal pursuit (**A**) and visually induced saccades (**B**). Discharge of this neuron is shown during corrective saccades (**C**) and oscillation (**D**).

sence of "foveation periods" in the monkey's oscillations suggests similarity more so to acquired, rather than congenital, pendular nystagmus. Our finding that gabapentin suppressed the oscillations (Figs. 3 and 4) is also consistent with the clinical observation that acquired pendular nystagmus is suppressed by gabapentin.[9,15,16]

Possible Neural Basis of Ocular Oscillation Resembling Pendular Nystagmus

Several hypotheses have been advanced for the neural mechanisms underlying the various forms of pendular nystagmus (see Introduction). The behavioral analysis of the present study shows that although oscillatory eye movements were observed both in the direction of pursuit eye movements and in the orthogonal direction, the peak-to-peak velocities and amplitudes of the oscillatory eye movements were significantly larger in the pursuit direction (Fig. 2). This suggests a contribution of smooth-pursuit neural pathways to the oscillatory eye movements.

Comparison of the peak velocity-amplitude relationship of the oscillatory eye movements when compared to small saccades (Figs. 5–6) suggests that the neurologic origin of the oscillatory eye movements is different from that of saccades. However, our neuronal recordings from the cerebellar floccular region clearly indicate some common contributing factor across corrective saccades and oscillatory eye movements, since those neurons responded during both types of eye movements (Table 2, group 1 and 2 neurons; Figs. 7–9). In particular, the group 2 neurons exhibited burst-tonic discharge during visually guided saccades, similar to the discharge of brain stem burst-tonic neurons considered to be a component of the neural integrator.[1] Axons from some of those brain stem neurons have been reported to project to the floccular region, and that may be the source of our group 2 burst-tonic signals, suggesting a contribution from the brain stem neural integrator to oscillatory eye movements, as proposed by Das et al.[4] Our present analysis seems to support some contribution from each of two competing hypotheses[3,4] regarding the neural mechanisms of pendular nystagmus.

Oscillation-related neurons (Table 2) were encountered within the molecular layer and often only tens of microns distant from complex-spike identified Purkinje cells. In the granular layer, only a small minority of oscillation-related non-Purkinje cells were encountered, as identified by complete absence of complex spike activity, and amid the neural "quiet" expected around only small ($\leq$10 μm), sporadically active granule cells.[26] Thus, a majority of the group 2 neurons are hypothesized to be basket and/or stellate cells, judging from the discharge characteristics and their location in the molecular layer.[26,27] If true, this suggests that oscillation-related oculomotor signals are sent to the cerebellar flocculus but are there "filtered" out by inhibitory, molecular layer interneurons, with oscillation signaling absent from flocculus region (i.e., Purkinje cell) output. However, our negative results do not preclude a contribution of pursuit-related Purkinje cells in other cerebellar areas, for example, the dorsal vermis, to oscillation.[1]

To understand the neural basis of oscillation further, we have developed an efference-copy pursuit model for analytical purposes.

A Model of Putative Pendular Nystagmus during Smooth Pursuit

Ocular oscillations are a common feature of smooth-pursuit onset and have been an important feature in the development of models for smooth pursuit.[28] A possible cause of the temporary oscillatory nystagmus in juvenile monkeys is a lack of development of the efference copy pathway (Fig. 10A) that is thought to provide damping of oscillations in pursuit.[3] Our hypothesis is that young animals probably have early development of a basic optokinetic/ocular following reflex[29] but initially lack the efference copy loop necessary for pursuit, developing only it as a result of the specific pursuit training. Our assumption is that open-loop gain of ocular following is initially quite low (~1) and that attempts to increase it to improve closed-loop pursuit gain create instability because of delays (~60–80 ms) in visuomotor processing. In humans it is known that even small moving targets can drive low-gain smooth eye movements reflexively, but that active participation is required to raise the gain for pursuit of a selected target.[30–33]

Development of the efference copy pathway effectively increases the gain of pursuit as well as stabilizing it.[34] Consequently, once it is established, high levels of open-loop gain are no longer required to achieve accurate and stable pursuit. The effect is illustrated in Figure 9B. In the absence of efference copy ($\beta = 0$) an open-loop gain (K) of 6 would achieve a closed-loop gain of ~0.75, but superimposed on the constant velocity response is an oscillation with peak velocity of ±37 deg/second at 3.88 Hz. In contrast, once efference copy is developed ($\beta = 0.95$), an open-loop gain (K) of only 2 yields a closed-loop gain of 0.98, and although there is an initial oscillation at 3.69 Hz it decays very quickly. Changes in closed-loop gain, amplitude, and frequency of oscillation with changes in β and K are shown in Figs. 10C–E, respectively, for a delay of 80 ms. The experimental results indicate that in the learning stage of pursuit, gain fluctuates quite widely from cycle to cycle (Fig. 4, baseline), and when gain is high there is, on average, an increase in oscillation. The effect of gabapentin may be to inhibit attempts to generate high gain and thereby reduce oscillation (Fig. 4, red).

Although it is not difficult to offer an explanation for oscillation in the primary axis of target motion, it is more difficult to suggest why there might be oscillation in the orthogonal axis. One possibility is that when gain is increased for a selected target it is not exclusive to one axis, but applies generally. If the gain is elevated in the orthogonal axis it would become potentially unstable in that axis, and any brief perturbation would set off the oscillation, which would not be damped in the absence of the efference copy pathway. Perturbations in the orthogonal axis could result from inaccurate directional control in inexperienced juvenile animals.

It should be noted, however, that models for pendular nystagmus that incorporate instability in the neural integrator

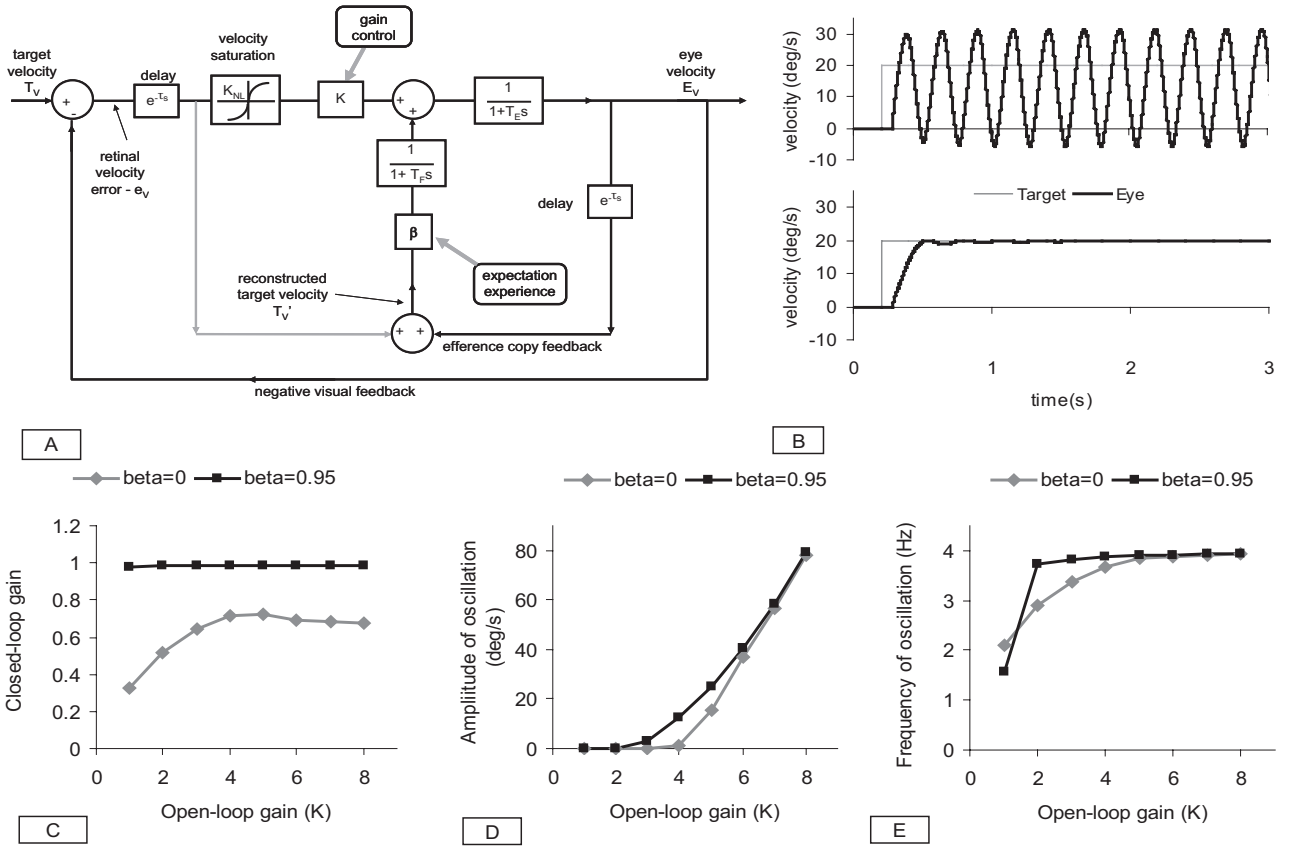

Figure 10. (**A**) A model of ocular pursuit in which simple negative feedback is supplemented by efference copy feedback. The efference copy signal is assumed to be summated with retinal velocity error to obtain an internal representation of target velocity (T_V'). Output from the efference copy loop is modified by expectation and experience, which regulates gain β and is low-pass filtered to increase stability. $T_E = 0.12$ second; $T_F = 0.12$ second; $\tau = 0.08$ second. (**B**) Comparison of attempts to achieve high closed-loop gain pursuit either (**A**) without efference copy ($\beta = 0$; $K = 6$) or (**B**) with efference copy functioning effectively ($\beta = 0.95$; $K = 2$). (**C–E**). Effects of efference copy feedback ($\beta = 0$ [*gray traces*]; beta = 0.95 [*black traces*]) and changes in open-loop gain (K) on closed-loop gain (**C**), amplitude of oscillation (**D**) and frequency of oscillation (**E**). Note that calculated oscillation frequency is unreliable when amplitude of oscillation is very low (e.g., when $K = 1–2$).

might also be developed to account for our present findings, since we did occasionally encounter the phenomenon of a large saccade inducing a phase shift in the pendular oscillations (Fig. 6), which could be explained by "resetting" of the neural integrator for eye movements.[4] Furthermore, an oculomotor integrator that is configured as a distributed network group of neurons,[35,36] with different weightings for saccades and pursuit signals, could explain why pendular and saccadic movement components of the ocular oscillations are closely synchronized (Fig. 6). Finally, recent studies of infantile nystagmus have indentified genetic defects such as the FRMD7 mutation,[8] which may cause failure of normal neuronal growth and development,[37] thereby leading to potentially unstable neural circuits governing the control of gaze.

In summary, our results suggest that the transient ocular oscillations observed in juvenile monkeys during pursuit training could provide an animal model for pendular nystagmus. Both smooth-pursuit pathways and the neural integrator may contribute to these ocular oscillations. Analysis using an efference-copy pursuit model supports our interpretation.

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