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Directional asymmetry in vertical smooth-pursuit and cancellation of the vertical vestibulo-ocular reflex in juvenile monkeys

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Abstract

Young primates exhibit asymmetric eye movements during vertical smooth-pursuit across a textured background such that upward pursuit has low velocity and requires many catch-up saccades. The asymmetric eye movements cannot be explained by the un-suppressed optokinetic reflex resulting from background visual motion across the retina during pursuit, suggesting that the asymmetry reflects most probably, a low gain in upward eye commands (Kasahara et al. 2006). In this study we examined 1) whether there are intrinsic differences in the upward and downward pursuit capabilities and 2) how the difficulty in upward pursuit is correlated with the ability of vertical VOR cancellation. Three juvenile macaques that had initially been trained only for horizontal (but not vertical) pursuit were trained for sinusoidal pursuit in the absence of a textured background. In 2 of the 3 macaques, there was a clear asymmetry between upward and downward pursuit gains and in the time course of initial gain increase. In the third macaque, downward pursuit gain was also low. It did not show consistent asymmetry during the initial 2 weeks of training. However, it also exhibited a significant asymmetry after 4 months of training, similar to the other 2 monkeys. After 6 months of training, these two monkeys (but not the third) still exhibited asymmetry. As target frequency increased in these 2 monkeys, mean upward eye velocity saturated at $\sim 15^\circ/\text{s}$, whereas horizontal and downward eye velocity increased up to $\sim 40^\circ/\text{s}$. During cancellation of the VOR induced by upward whole body rotation, downward eye velocity of the residual VOR increased as the stimulus frequency increased. Gain of the residual VOR during upward rotation was significantly higher than that during horizontal and downward rotation. The time course of residual VOR induced by vertical whole body step-rotation during VOR cancellation was predicted by addition of eye velocity during pursuit and $\text{VOR} \times 1$. These results support our view that the directional asymmetry reflects the difference in the organization of the cerebellar floccular region for upward and downward directions and the preeminent role of pursuit in VOR

cancellation.

Introduction

The smooth-pursuit system has evolved in primates to maintain the image of an object of interest on the foveae of both eyes during movement. During whole body rotation, the smooth-pursuit system does not work independently but interacts with the vestibular system (see Leigh and Zee 2006 for a review). Recently, our laboratory has shown that young (but not mature) primates (Japanese macaques and human children) produced asymmetric eye movements during vertical pursuit across a textured background such that upward pursuit had low velocity and required many catch-up saccades (Takeichi et al. 2003). In addition, VOR cancellation was severely impaired during upward gaze movements when young monkeys were required to cancel the downward VOR by tracking a target that moved upward in space together with upward whole body rotation. Further studies have indicated that the low-velocity, upward pursuit eye movements of Japanese macaques cannot be explained by the un-suppressed downward optokinetic reflex triggered by the background visual motion across the retina during pursuit eye movements, suggesting that the directional asymmetry reflects most probably, the low gain in upward pursuit eye commands (Kasahara et al. 2006).

In the laboratory, it is necessary to train all tested monkeys for some period in order to ensure excellent pursuit. In other words, smooth-pursuit eye movements with the gains of ~ 0.9 are learned through training. The high level of training might have obscured any possible differences between upward and downward pursuit capabilities in the absence of challenges such as pursuit against a textured background. We sought to determine whether there are intrinsic differences in the upward and downward pursuit capabilities by using juvenile monkeys with little training. Previous studies were unable to answer this question, because all the young monkeys tested had been well trained for several months until they were able to perform smooth-pursuit of a small spot moving vertically

and horizontally in the absence of a textured background (Takeichi et al. 2003; Fukushima et al. 2003; Kasashara et al. 2006). In the first series of the present study, we asked this question for vertical pursuit in juvenile macaques that had initially been trained only for horizontal (but not vertical) pursuit. We then tested the time course of gain increase in upward and downward pursuit eye movements in the absence of a textured background.

Neural mechanisms of VOR cancellation remain still controversial (see Leigh and Zee 2006 for a review). It has been argued that the two neural mechanisms are used during VOR cancellation: the addition mechanism and the parametric adjustment mechanism (Lisberger 1990). In the addition mechanism, the pursuit system and the VOR operate entirely independently and the two signals sum or cancel each other (also Misslisch et al. 1996). The parametric adjustment mechanism is a non-pursuit mechanism, and there is a momentary adjustment of transmission in the VOR pathways to suppress the VOR itself (McKinley and Peterson 1985; Lisberger 1990; Roy and Cullen 1998; Belton and McCrea 2000; also Takeichi et al. 2000). Our monkeys with upward and downward pursuit asymmetry provide an excellent opportunity to examine the contribution of smooth-pursuit to the cancellation of the VOR. If the properties of cancellation of the VOR correlate closely with the unique properties of smooth-pursuit, we can conclude that pursuit is a major contributor. Therefore, in our second series we compared the frequency response of smooth-pursuit and VOR cancellation during sinusoidal target and/or whole body rotation and also examined the time course of VOR cancellation using whole body step-rotation to assess whether the addition of vestibular and pursuit mechanism can explain VOR cancellation.

Materials and Methods

By careful examination of tooth eruption, Mouri (1994) reported that male adolescent growth starts when Japanese macaques reach 4 years old and that full eruption of all the permanent teeth occurs

during their sixth year. Female reproductive ability is reported to be complete at 8 years old. The three male Japanese monkeys we tested in this study were 4 years old (*Macaca fuscata*, S, Y, J, 3.0-3.6 kg). Comparison of the body weights and dental ages of these monkeys with those of previous studies suggests that the monkeys used in the present study were younger than the previous monkeys (3.75-4.5 kg in Takeichi et al. 2003; 4.0-5.5 kg in Kasahara et al 2006). Our experimental protocols were approved by the Animal Care and Use Committee of Hokkaido University School of Medicine. The general methods for animal preparation, training, eye movement recording, and data analysis were described in detail previously (Takeichi et al. 2003; Kasahara et al. 2006) except for the specific training methods used for pursuit during the initial training in this study.

Briefly, each monkey was sedated with ketamine hydrochloride (5 mg/kg, i.m.), and then anesthetized with pentobarbital sodium (25 mg/kg, i.p.). Under aseptic conditions, head holders were installed to restrain the head firmly in the primate chair in the stereotaxic plane. Vertical and horizontal components of eye movements were recorded by the scleral search coil method (Fuchs and Robinson 1966; Judge et al. 1980). Analgesics and antibiotics were administered post-surgically to reduce pain and prevent infection. Following a week of recovery, the monkeys were trained with apple juice reward for tracking a target spot (0.5° in diameter) on a 21 inch computer monitor (120 Hz rate) 60 cm in front of the animals' eyes in an otherwise dark enclosure. Monkeys' heads were firmly restrained in the primate chair in the stereotaxic plane. The monkey chair was fixed to the turntable that had two degrees of freedom of motion (horizontal and vertical rotation) under computer control. The inter-aural midpoint of the animals' head was brought close to the axis of vertical and horizontal rotation.

Training and recording procedures, and behavioral paradigms

The differences in training method between the present study and our previous one (e.g., Takeichi

et al. 2003) are that during the initial training, which lasted for 3-5 days and ~30 min per day, our monkeys were trained only in horizontal pursuit at 0.2 Hz ($\pm 10^\circ$, peak velocity 12.6°/s). Reward circuits compared target position signals with the monkeys' eye position signals. If the monkeys' gaze was within the error window of $\pm 5^\circ$ for 0.5 s, a drop of apple juice was automatically delivered to the monkeys. In the first series of the present experiments, we set the reward window wider compared to the previous studies (e.g., Kasahara et al. 2006, $\pm 1^\circ$ for 0.5-1 s) in order to study pursuit eye movements from the very beginning of training. This was to allow the inexperienced monkeys to obtain reward for their poorer tracking. Once the gain of horizontal pursuit reached 0.7, we started training the monkeys in vertical pursuit for ~2 weeks at 0.2 Hz ($\pm 10^\circ$), ~30 min per day. Eye position signals were calibrated by requiring the monkeys to make visually guided saccades in darkness to a target of known eccentricity ($\pm 10^\circ$) horizontally and vertically. We examined whether there was a difference in the time course of upward and downward pursuit gain changes in the absence of a textured background. Horizontal pursuit was also tested to examine further the effects of training. Because all three monkeys tested could perform horizontal pursuit reasonably well at the start of vertical pursuit training (gain ≥ 0.7), we assumed that the monkeys understood the task and any difference in the time course of gain changes in upward and downward directions during the initial vertical pursuit training reflected a difference in learning. After their initial vertical pursuit training, these monkeys were trained in other pursuit eye movement tasks. Gains of horizontal and vertical pursuit were tested again 4 months later in 3 monkeys at 0.2 Hz ($\pm 10^\circ$).

During 6-8 months after the initial training, two (S, Y) monkeys but not J still exhibited asymmetry between upward and downward pursuit in the absence of a textured background. In the second series of the present study, they were again tested for vertical and horizontal smooth-pursuit at a variety of frequencies (0.1-1.0 Hz, $\pm 10^\circ$) to examine frequency response of smooth-pursuit as

described previously for adult monkeys (e.g., Fukushima et al. 2000). Whole body sinusoidal rotation was also applied either horizontally or vertically at a variety of frequencies (0.1-1.0 Hz, $\pm 10^\circ$) while requiring the monkeys to track a target that moved in space with the same amplitude, direction and phase as the chair rotation. This task required the monkeys to cancel the VOR so that the eyes remained relatively motionless in the orbit and gaze moved with the chair. Typically 20 cycles were repeated for each frequency.

To examine the time course of VOR cancellation in the two (S, Y) monkeys, whole body step-rotation ($20^\circ/\text{s}$ for 1 s, peak acceleration $\sim 600^\circ/\text{s}^2$) was also applied either vertically or horizontally with random inter-trial intervals of 1-3.5 s as reported previously (e.g., Akao et al. 2007). Target position was controlled using the chair position signal obtained from a potentiometer attached to the axis of the turntable. For comparison, the target stayed stationary in space during chair rotation and the monkeys were required to fixate the stationary spot by a perfect ($\times 1$) VOR so that gaze remained stationary in space (VOR $\times 1$). Smooth-pursuit was also tested by moving the target alone in the identical ramp trajectory at $20^\circ/\text{s}$ (for 1 s) without chair rotation. Each task condition was tested as a block. Typically 20 trials were tested for each of horizontal and vertical directions. In these two monkeys, experiments for frequency response of smooth-pursuit and VOR cancellation and whole body step-rotation were repeated on different days to calculate overall means for each monkey. The third monkey (J) that no longer exhibited directional asymmetry after 6 months of training was used for some other experiments.

Figure 1 near here

Data analysis

Eye-, target-, and chair- position signals were digitized at 500 Hz using a 16-bit A/D board (National Instruments) on a Macintosh Quadra computer. These position signals were differentiated by analogue circuits (DC-100 Hz, -12 dB/octave) to obtain velocity. Saccades were identified and

removed using the interactive computer program utilizing a maximum likelihood ratio criterion (Singh et al. 1981; Fukushima et al. 2000).

During the initial pursuit training, our monkeys often exhibited oscillatory eye movements (Fig. 1b, arrows). During normal vertical pursuit (Fig. 1a), horizontal eye velocity records (third trace) exhibited only saccades whose occurrences were synchronized with vertical corrective saccades (second trace). However, during attempted vertical smooth-pursuit, intrusive horizontal oscillatory eye movements appeared (Fig. 1b, HE vel, HE pos, arrows). Careful observation using an infrared camera suggests that they were not due to the loss of alertness. These oscillatory eye movements had peak velocities of 5-6 °/s with frequencies of 3-4 Hz (Fig. 1b, HE vel, VE vel). Because the exact nature of the eye movements is unknown, we did not include these eye movement data in the present analysis and only those records in which such eye movements did not appear during pursuit were accepted for further analysis.

To examine the initial training effects, typically 300 traces were aligned with stimulus velocity to obtain means and standard deviations (SD) for each day. As reported previously, vertical pursuit of young monkeys exhibited an asymmetry (e.g., Fig. 1d, also Takeichi et al. 2003; Kasahara et al. 2006). For asymmetric eye velocity responses during sinusoidal vertical pursuit, we measured peak eye velocity for upward and downward components separately by manually positioning a horizontal cursor on the peak of the mean eye velocity trace on the computer monitor. SDs were similarly measured. Then, by dividing these values by peak target velocity, mean ($\pm$ SD) gains for upward and downward eye velocity were calculated separately. Mean ($\pm$ SD) gains for horizontal (leftward and rightward) eye velocity were calculated similarly for consistency. However, because there was no asymmetry in the horizontal eye velocity (e.g., Fig. 1c), horizontal eye velocity gain was calculated by averaging the leftward and rightward eye velocity gains.

To analyze frequency response of smooth-pursuit and VOR cancellation, typically 20 traces were aligned with stimulus velocity to obtain means and SD. Mean $\pm$ SD eye velocity in each

direction was measured separately as described above. Mean $\pm$ SD gains for upward and downward eye velocity were calculated separately. During horizontal pursuit and VOR cancellation, rightward and leftward eye velocities were similar and they were averaged. Frequency response of VOR cancellation during whole body rotation was plotted as eye velocity and gain (re stimulus velocity) of residual VOR against chair frequency. To examine the time course of VOR cancellation, eye velocity of residual VOR induced by whole body step-rotation was superimposed with respect to the stimulus onset. For comparison, eye velocity during smooth-pursuit induced by identical target motion trajectory and eye velocity during VOR x1 induced by whole body step-rotation were also superimposed. Mean $\pm$ SD eye velocity in each condition was then compared. To measure latencies of eye movements, the control values of eye velocity (mean $\pm$ SD) were calculated from the 200 ms interval immediately before the stimulus onset. Latencies of eye movements induced by whole body step-rotation and/or step target-motion were assessed as the time at which mean eye velocity deviated mean + or - 2.0 SD of the control value as described previously (Akao et al. 2004).

Figure 2 near here

Results

Up down asymmetry in smooth-pursuit

Figure 2 (a-c) summarize the time course of mean ($\pm$ SD, shaded) gain changes for horizontal and vertical (upward and downward) pursuit in three monkeys. There was individual variability in their performance. For example, monkey S (Fig. 2a) exhibited initial horizontal pursuit gain (filled circles) of 0.4, and after about 10 days of training the mean horizontal gains increased to 0.8. For vertical pursuit, this monkey exhibited a clear asymmetry in upward (open squares) and downward (triangles) eye velocity gains in the first day of vertical pursuit training. Downward mean eye velocity gain was 0.6 and increased to $\sim$ 1 within a week, but upward eye velocity gain was only 0.1, and even after a week of training it was still less than 0.4. Monkey Y (Fig. 2b) also exhibited higher

gains for downward eye velocity (mean ~ 0.5) than upward eye velocity (mean ~ 0.2) during the initial training and showed a similar time course for gain changes of horizontal pursuit and upward- and downward- pursuit.

In contrast, for monkey J (Fig. 2c) the initial horizontal pursuit gain was 0.7 and remained relatively constant during the 15 days of training. Downward mean eye velocity gain was only 0.2-0.3, much lower than that in the other two monkeys. Although upward pursuit gains were slightly higher than downward pursuit gains during the first and second training days of vertical pursuit (Fig. 2c, *), the difference was not consistently observed during the initial two weeks of training. For initial upward pursuit gains in Fig. 2 (a-c), we fit a least-squares, straight line to compare the change in gain increase between the three monkeys. The slopes were similar in all three monkeys tested, and averaged 0.04 per day (Fig. 2a-c).

Figure 2 (a-c, symbols on the right) summarizes mean ($\pm$ SD) eye velocity gains after 4 months using identical pursuit task conditions (see Materials and Methods). All three monkeys exhibited consistent results. Gains of horizontal pursuit and downward pursuit were similar and were ~ 0.9 , whereas upward pursuit gains were 0.4-0.5, approximately half the downward pursuit gains, even without the presence of a textured background (cf. Takeichi et al. 2003; Kasahara et al. 2006).

Figures 3 and 4 near here

Frequency response of smooth-pursuit eye movements

In the second series of experiments, vertical and horizontal smooth-pursuit eye movements were tested at 0.1-1.0 Hz ($\pm 10^\circ$) in two monkeys (S, Y) to examine frequency response of smooth-pursuit eye movements (see Materials and Methods). The results were similar in the two monkeys. For monkey S, Fig. 3 (a, b) plot mean ($\pm$ SD) eye velocity and gain of horizontal, upward and downward eye velocity against target frequency. As target frequency increased from 0.1 to 0.7 Hz, both horizontal and downward mean eye velocity increased up to ~ 35 - 40° /s (Fig. 3a) with gains above

0.8 (Fig. 3b). Only at 1.0 Hz, mean gains of horizontal and downward eye velocity decreased to 0.6-0.8 (Fig. 3b). In contrast, upward mean eye velocity (open squares) saturated at $\sim 15^\circ/\text{s}$ (Fig. 3a). Mean gains of upward pursuit were ≤ 0.4 at target frequencies 0.5-1.0 Hz (Fig. 3b). Asymmetry in upward and downward pursuit gain was evident at all frequencies tested (Fig. 3b).

Frequency response of VOR cancellation

VOR cancellation was tested in two monkeys (S, Y) at the same frequencies as smooth-pursuit (0.1-1.0 Hz, $\pm 10^\circ$) to compare the frequency response of smooth-pursuit eye movements and VOR cancellation (see Materials and Methods). The results were similar in the two monkeys. For monkey S, Fig. 3 (c, d) plot mean ($\pm$ SD) eye velocity and gain of residual VOR during horizontal, upward and downward chair rotation against target/chair frequency. Eye velocity and gain of residual VOR during horizontal and downward VOR cancellation exhibited similar results. At 0.1- 0.5 Hz, mean eye velocities of residual VOR were minimal (Fig. 3c). Only at 0.7 and 1.0 Hz, eye velocities slightly increased (Fig. 3c). Mean gains were low (<0.3 , typically <0.2 , Fig. 3d).

In contrast, during upward VOR cancellation, downward eye velocity and gain of residual VOR were significant (Fig. 3c, d). Downward eye velocity of the residual VOR increased almost linearly up to $\sim 45^\circ/\text{s}$ (Fig. 3c), and gain also increased from ~ 0.5 to 0.7 (Fig. 3d). Asymmetry in gain between upward and downward VOR cancellation was evident at all frequencies tested (Fig. 3d). Thus, the low eye velocity gain during upward smooth-pursuit (Fig. 3b) and the high gain of residual VOR during upward VOR cancellation (Fig. 3d) are closely correlated.

The time course of vertical VOR cancellation

To elucidate how the asymmetry in downward and upward smooth-pursuit was linked with the asymmetry in the performance during VOR cancellation (Fig. 3), we examined the time course of VOR cancellation during vertical whole body step-rotation (at $20^\circ/\text{s}$ for 1s) (see Materials and

Methods). The results were similar in two monkeys (S, Y). Figure 4 summarizes mean ($\pm$ SD) eye velocity of monkey S. Downward whole body step-rotation (Fig. 4a, vertical line with upward arrow) induced upward VOR with latencies ~ 10 ms (black and red, VOR x1 and VOR cancellation, respectively). Initial upward VOR exhibited the first peak in eye velocity at 70 ms (Fig. 4a, open arrow) followed by the second peak at 110 ms (vertical line). Eye velocities at the first peak during the two VOR task conditions were similar and averaged $21^\circ/\text{s}$. The average eye velocity at the second peak (Fig. 4a, vertical line) was smaller during VOR cancellation compared to the peak during VOR x1 (red vs black). During VOR cancellation, upward eye velocity decreased sharply after the second peak, with a time course (Fig. 4a, red) similar to that of downward smooth-pursuit induced by the identical target trajectory (Fig. 4a, blue).

Upward whole body step-rotation (Fig. 4b, vertical line with upward arrow) induced downward VOR with latencies ~ 10 ms (black and red, VOR x1 and VOR cancellation, respectively). Initial downward VOR exhibited the first eye velocity peak at 70 ms (Fig. 4b, open arrow) followed by a second peak at 110 ms (vertical line) during the two vestibular task conditions. Eye velocities at the first peak were similar during VOR cancellation (Fig. 4b, red, $21^\circ/\text{s}$) and VOR x1 (black, $22^\circ/\text{s}$). The eye velocity at the second peak (Fig. 4b, vertical line) was slightly smaller during VOR cancellation compared to the peak during VOR x1 (red vs black). During VOR cancellation (Fig. 4b, red), the decrease of downward eye velocity after the second peak was small, with the time course (Fig. 4b, red) similar to that of upward smooth-pursuit induced by the identical target trajectory (Fig. 4b, blue). Latencies of smooth-pursuit eye movements were ~ 110 ms (Fig. 4a, b).

To test whether residual VOR during VOR cancellation was explained by addition of eye velocity during smooth-pursuit and VOR x1, we compared actual eye velocity during VOR cancellation (Fig. 4a, b, red) with predicted eye velocity (mean $\pm$ SD) that was the sum of eye velocity during smooth-pursuit and VOR x1 (Fig. 4a, b, green). The predicted eye velocity during

downward target/chair motion was nearly identical to the actual eye velocity during the initial responses (Fig. 4a, green vs red). The actual mean eye velocity during upward target/chair motion (Fig. 4b, red) was slightly smaller than the predicted mean eye velocity (Fig. 4b, green) especially during the time interval between the first peak eye velocity (before the onset of smooth-pursuit) and second peak eye velocity (near the onset of smooth-pursuit). However, their SDs overlapped each other and the difference was not significant. These results indicate that the eye velocity during the VOR cancellation task can be explained primarily by addition of eye velocity during smooth-pursuit and $VOR \times 1$.

Discussion

The present results extend previous findings (Takeichi et al. 2003; Kasahara et al. 2006) by showing that, in the absence of a textured background, there is a clear asymmetry between upward and downward smooth-pursuit gains, as well as in the time course of initial gain increase, between upward and downward directions in two of the three monkeys tested (Fig. 2a-c). Although consistent asymmetry was not observed in the third macaque (J) during the initial two weeks of training because downward pursuit gain was also low, this monkey also exhibited a significant asymmetry after 4 months, similar to the other two monkeys (Fig. 2c). The reason for the individual variability is unknown. Nevertheless, these results indicate that there are intrinsic differences in the upward and downward pursuit capabilities in juvenile monkeys with little training.

The present findings are consistent with previous observation showing that human children (8-10 years old) exhibit upward/downward pursuit asymmetry in darkness without a textured background (Takeichi et al. 2003). It should be noted that in previous studies (Takeichi et al. 2003; Kasahara et al. 2006), the monkeys were extensively trained and the up/down asymmetry during vertical pursuit against a homogenous background was negligible. In the present study the up/down asymmetry became negligible after 6 months of training in one of the 3 monkeys tested. However,

the remaining 2 monkeys still exhibited the asymmetry even after 8 months of training. We do not know the exact reason why it took such a long time for the two monkeys to compensate for the asymmetry in the absence of a textured background. We think that the age of the monkeys as well as training are important factors that determine pursuit capabilities. In previous studies, ages of the monkeys were unknown and we estimated their ages from eruption of the permanent teeth. It is also known that body weights are closely related with ages in young monkeys (Mouri 1994). Body weights of 3 monkeys used in the present study (3.0-3.6 kg) were lighter than those of the monkeys used in previous studies (3.75-4.5 kg in Takeichi et al. 2003; 4.0-5.5 kg in Kasahara et al 2006). Comparison of body weights and dental ages in these studies suggests that the monkeys used in the present study were youngest.

The present study also provides further details concerning the upward and downward pursuit asymmetry. Compared to horizontal and downward pursuit eye velocity that increased up to $\sim 40^\circ/\text{s}$, upward eye velocity saturated at $\sim 15^\circ/\text{s}$ (Fig. 3a). Moreover, the vertical asymmetry in the two monkeys was linked with the asymmetry in the monkeys' ability to cancel the vertical VOR (Figs. 3, 4). In particular, the latency of the onset of VOR cancellation following the onset of whole body step-rotation was consistent with the latencies of smooth-pursuit (Fig. 4). The time course of eye velocity of the residual VOR during whole body step-rotation was predicted by linear addition of eye velocity during smooth-pursuit and $\text{VOR} \times 1$ in both upward and downward whole body step-rotation (Fig. 4). Notice that in our task condition there was no clear asymmetry in the initial components of the VOR induced by downward and upward whole body step-rotation (Fig. 4a, b, open arrows; also Takeichi et al. 2003). Previous studies examined *horizontal* smooth-pursuit and VOR cancellation using the identical stimulus trajectory (Akao et al. 2007); the latency and the time course of eye velocity of the residual VOR during VOR cancellation was also predicted by linear addition of eye velocity during smooth-pursuit and $\text{VOR} \times 1$.

Two neural mechanisms have been used to explain VOR cancellation (see Introduction): the addition mechanism and the parametric adjustment mechanism (Lisberger 1990). Previously, we have shown that pursuit signals and vestibular signals add linearly in pursuit neurons in the caudal part of the frontal eye fields during cancellation of the VOR induced by whole body step-rotation (Akao et al. 2007). The study by Mustari et al. (2003) also documents asymmetry of horizontal smooth-pursuit and asymmetry in cancellation of the VOR following unilateral muscimol inactivation of the dorsolateral pontine nucleus. These observations together with the present results suggest that VOR cancellation is mostly performed by the addition mechanism in our task conditions. Lisberger (1990) pointed out that parametric modulation of the VOR is a strategy that is invoked by monkeys voluntarily and whether a monkey employs parametric modulation of the VOR depends on training procedures, experimental conditions, and the level of motivation. We do not exclude the possibility that the parametric adjustment mechanism could also contribute during upward VOR cancellation if our monkeys had been trained more extensively (Fig. 4b, red *vs* green).

As the possible neural correlates, we have suggested that the directional asymmetry reflects the difference in the organization of the cerebellar floccular region and vestibular nuclei for upward and downward smooth eye movements (Kasahara et al. 2006; Fukushima et al. 2006 for a review). Briefly, preferred directions of the simple-spike discharge of the majority of pursuit Purkinje cells in the floccular region are either ipsiversive or downward (Miles et al. 1980; Stone and Lisberger 1990; Shidara and Kawano 1993; Krauzlis and Lisberger 1996; Fukushima et al. 1999). The difficulty in canceling the downward VOR during upward pitch rotation in young primates (Takeichi et al. 2003) may reflect the scarcity of upward floccular Purkinje cells that presumably inhibit downward eye velocity vestibular neurons (Zhang et al. 1995). Non-linearity in discharge modulation has been reported for horizontal Purkinje cells during off-direction pursuit (Stone and

Lisberger 1990; Lisberger et al. 1994). It is possible that such non-linearity also exists for downward Purkinje cell activity during upward pursuit. In our preliminary study, the two monkeys (S, Y) that exhibited asymmetry in vertical pursuit also exhibited asymmetry in upward and downward ocular following responses (Akao et al. unpub obs). The involvement of the floccular region in ocular following responses has been well established (Kawano 1999). These results taken together further suggest the involvement of the floccular region in the directional asymmetry in vertical pursuit.

The oscillatory eye movements observed in the present study during the initial pursuit training (Fig. 1b) suggest cross-coupling between vertical and horizontal slow eye velocity, which does not occur in normal pursuit (Fig. 1a). Although the exact mechanisms are unknown, these oscillatory eye movements may suggest inappropriate spatial (i.e., either horizontal or vertical), and temporal processing (i.e., gain) of target-motion signals into motor commands. As for the former, the untrained monkeys exhibited more variability in the direction of pursuit during the oscillation, so that vertical pursuit included substantial horizontal components (Fig. 1b). As for the latter, oscillations with similar frequencies (3-4 Hz) are known to occur during pursuit. Such oscillations may be intrinsic to the motor pathways for pursuit including the putative internal positive feedback loop (Robinson et al. 1986) or they may occur as the result of delays in the visual feedback control of pursuit (Goldreich et al. 1992). Further studies are needed to critically test the above-mentioned possibilities for the directional asymmetry observed in Japanese macaques and the neural correlates for the oscillatory eye movements.

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Figure legends

Fig. 1. Vertical and horizontal eye movements during initial pursuit training. **a**, normal vertical pursuit. **b**, peculiar eye movements (arrows) in horizontal and vertical components during vertical pursuit. HE pos and VE pos are horizontal and vertical eye position. HE and VE vel are horizontal and vertical eye velocity. **c** and **d** are superimposed normal horizontal and vertical pursuit for eye position and eye velocity traces. Saccade velocities are clipped in all velocity traces and are shown in grey. Saccades were also deleted in position traces in **c** and **d**. In **a-d**, superimposed thin dashed lines on eye position and velocity traces are target position and velocity trajectory, respectively, as indicated. Zero lines are shown by straight lines in vertical eye velocity traces (**a, b**) and horizontal and vertical eye velocity (**c, d**). In horizontal eye velocity traces in **a** and **b**, zero lines are indicated by thin dashed lines. All traces were obtained in day 5 after the start of vertical pursuit training.

Fig. 2. The time course of gain changes in horizontal and vertical pursuit during the initial training. **a, b, c**, mean $\pm$ SD gains for horizontal and upward and downward directions (indicated by keys) are illustrated for three monkeys after the start of horizontal pursuit training (day 0). The first points for upward and downward pursuit gains in each monkey are gains at the first day of vertical pursuit training. Points plotted to the right are mean ($\pm$ SD) gains after $\sim$ 4 months of pursuit training. For the initial upward pursuit gain increase, linear regressions were fit for each monkey as indicated (r =correlation coefficient).

Fig. 3. Frequency response of smooth-pursuit and VOR cancellation. **a** and **b**, mean $\pm$ SD eye velocity and gain are plotted against target frequency for horizontal, upward, and downward pursuit (indicated by keys). **c** and **d**, mean $\pm$ SD eye velocity and gain of residual VOR are plotted against target and chair frequency for horizontal, upward, and downward whole body rotation (indicated by keys). All data were taken from monkey S.

Fig. 4. Time course of vertical VOR cancellation and prediction of VOR cancellation by addition of eye velocity during smooth-pursuit and VOR x1. **a**, de-saccaded and averaged eye velocity (mean $\pm$ SD) during downward smooth-pursuit (SP, blue), upward mean $\pm$ SD eye velocity induced by downward whole body step-rotation (VOR x1, black) and by downward target motion and whole body rotation (VOR cancellation, red). Green line is predicted eye velocity during VOR cancellation by addition of eye velocity during downward smooth-pursuit and VOR x1 induced by downward step-rotation. **b**, de-saccaded and averaged eye velocity (mean $\pm$ SD) during upward smooth-pursuit (SP, blue), downward mean $\pm$ SD eye velocity induced by upward whole body step-rotation (VOR x1, black) and by upward target motion and whole body rotation (VOR cancellation, red). Green lines are predicted eye velocity during VOR cancellation by addition of eye velocity during upward smooth-pursuit and VOR x1 induced by upward step-rotation. In **b**, eye velocity trace during smooth-pursuit and predicted eye velocity are not continuous because of corrective saccades.

Fig. 1

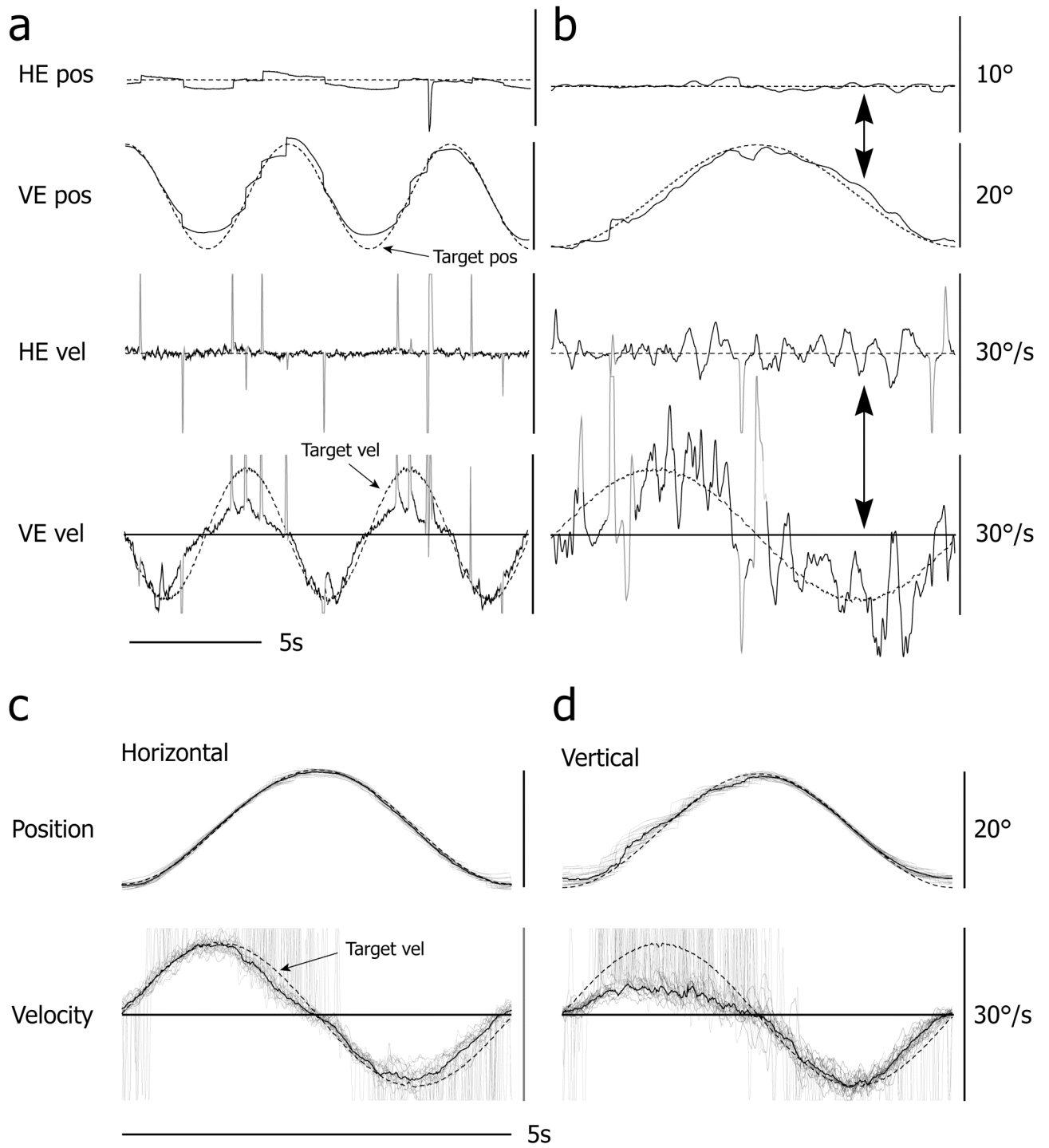


Fig. 2

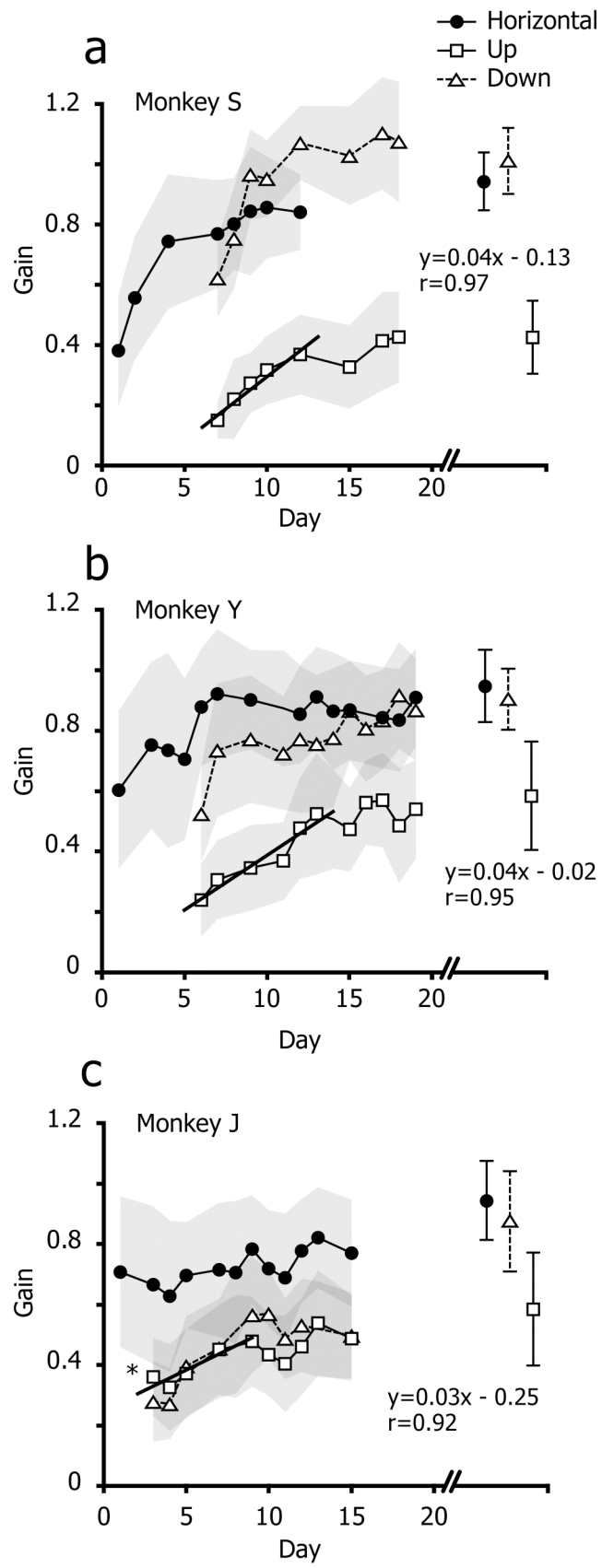


Fig. 3

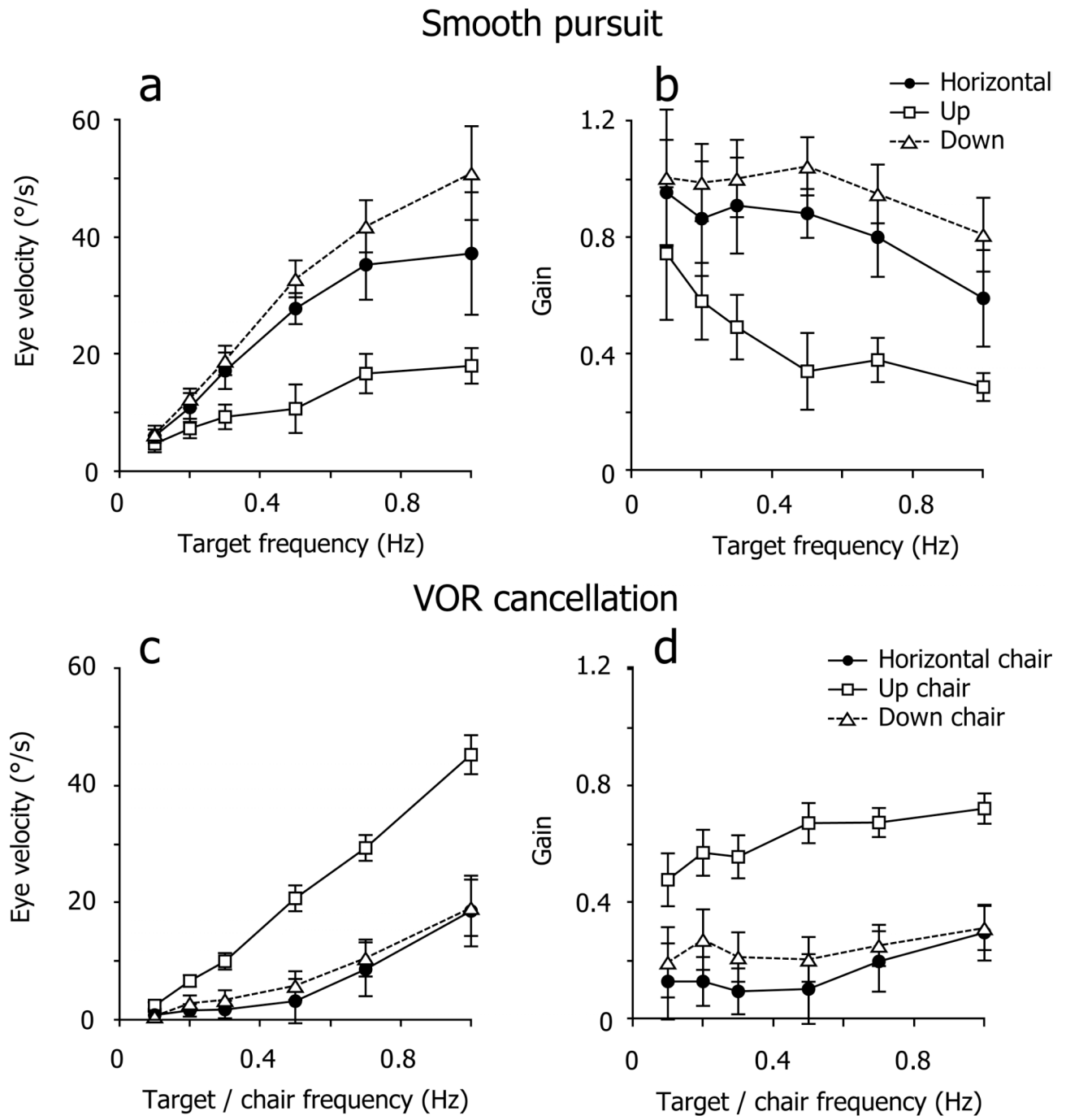


Fig. 4.

