Latency of vestibular responses of pursuit neurons in the caudal frontal eye fields to whole body rotation

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Abstract

The smooth pursuit system and the vestibular system interact to keep the retinal target image on the fovea by matching the eye velocity in space to target velocity during head and/or whole body movement. The caudal part of the frontal eye fields (FEF) in the fundus of the arcuate sulcus contains pursuit-related neurons and the majority of them respond to vestibular stimulation induced by whole body movement. To understand the role of FEF pursuit neurons in the interaction of vestibular and pursuit signals, we examined the latency and time course of discharge modulation to horizontal whole body rotation during different vestibular task conditions in head-stabilized monkeys. Pursuit neurons with horizontal preferred directions were selected, and they were classified either as gaze-velocity neurons or eye/head-velocity neurons based on the previous criteria (Fukushima et al. 2000). Responses of these neurons to whole body step-rotation at 20°/s were examined during cancellation of the vestibulo-ocular reflex (VOR), VORx1, and during chair steps in complete darkness without a target (VORd). The majority of pursuit neurons tested (~70%) responded during VORd with latencies < 80 ms. These initial responses were basically similar in the three vestibular task conditions. The shortest latency was 20 ms and the modal value was 24 ms. These responses were also similar between gaze-velocity neurons and eye/head-velocity neurons, indicating that the initial responses (< 80ms) were vestibular responses induced by semicircular canal inputs. During VOR cancellation and x1, discharge of the two groups of neurons diverged at ~90 ms following the onset of chair rotation, consistent with the latencies associated with smooth pursuit. The shortest latency to the onset of target motion during smooth pursuit was 80 ms and the modal value was 95 ms. The time course of discharge rate difference of the two groups of neurons between VOR cancellation and x1 was predicted by the discharge modulation associated with smooth pursuit. These results provide further support for the involvement of the caudal FEF in integration of vestibular inputs and pursuit signals.

Introduction

The oculomotor smooth-pursuit system in primates keeps the retinal target image on the fovea by matching the eye velocity to target velocity. During head and/or whole body movement, this system is coordinated with the vestibular system to maintain the accuracy of eye-movements in space (see Leigh and Zee 1999 for a review). The caudal part of the frontal eye fields (FEF) in the fundus of the arcuate sulcus contains many smooth-pursuit related neurons (pursuit neurons) (e.g., MacAvoy et al. 1991; Gottlieb et al. 1993, 1994; Tanaka and Fukushima 1998). Using sinusoidal whole body rotation in the presence of a pursuit target, previous studies in our laboratory have shown that virtually all FEF pursuit neurons tested responded to vestibular stimulation (Fukushima et al. 2000). Specifically, by comparing discharge modulation during two task conditions (i.e., VOR cancellation and VOR x1, cf., Lisberger and Fuchs 1978), FEF pursuit neurons can be classified either as gaze-velocity neurons or eye/head-velocity neurons. During the VOR cancellation task, the monkeys were required to cancel the VOR by tracking a target that moved in space with the same amplitude, direction and phase as the chair rotation so that the eyes remained virtually motionless in the orbit and gaze moved with the chair. In the VOR x1 task, the monkeys fixated the stationary spot during whole body rotation by a perfect (x1) VOR and gaze remained stationary in space. Pursuit neurons that exhibited stronger discharge modulation during VOR cancellation than x1 were classified as gaze-velocity neurons, whereas most eye/head-velocity neurons exhibited opposite discharge characteristics. Furthermore, the majority of tested neurons in both groups (28/43=~65%) responded to chair rotation in complete darkness without a target, suggesting the vestibular origin of their responses (Fukushima et al. 2000).

To understand the role of caudal FEF pursuit neurons in the interaction of vestibular and pursuit signals, important questions still remain. First, information about the latency of vestibular responses of pursuit neurons is necessary. However, latencies of vestibular responses in the caudal FEF were

examined previously only by the evoked potential method following electrical stimulation of the labyrinth in anesthetized monkeys (Ebata et al. 2004). These authors, using the laminar field potential analysis, suggested that vestibular-evoked potentials in the caudal FEF were directly conveyed to the cortex through the thalamus (Ebata et al. 2004). In the caudal FEF of alert monkeys, vestibular-related discharge modulation is observed not only in pursuit neurons but also in neurons that are not related to pursuit eye movements (Fukushima et al. 2000). In this study we examined latencies of vestibular responses of identified pursuit neurons in the caudal FEF to whole body, step-rotation. Second, information about the time course of discharge modulation of pursuit neurons during different vestibular task conditions is necessary to understand how vestibular and pursuit signals interact in the caudal FEF. For this, we compared the time course of discharge modulation of the two groups of neurons during VOR cancellation and x1.

Our results show that vestibular signals reach the majority of FEF pursuit neurons with latencies of 20-24 ms after the onset of whole body step-rotation. The initial vestibular responses (<80 ms) that were basically similar in gaze-velocity neurons and eye/head-velocity neurons diverged at ~90 ms during VOR cancellation and x1, consistent with the latencies associated with smooth pursuit. The time course of discharge rate difference between the two tasks was predicted by the discharge modulation associated with smooth pursuit. Thus, the present results provide further support for the involvement of the caudal FEF in the interaction of vestibular inputs and pursuit signals.

Materials and Methods

Data were collected from three male Japanese monkeys (C, SI, SH, Macaca fuscata, 3.5-5.5 kg, 4-6 years old) that were subjected to whole body, step-rotation with a trapezoidal trajectory (e.g., Tsubuku et al. 2006) while recording from pursuit neurons in the caudal FEF. 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, vestibular stimulation, recording, and data analysis were described in detail

previously (Fukushima et al. 2000, 2001a; Akao et al. 2005). 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).

Extracellular recordings of pursuit neurons were made mostly in the fundus of the arcuate sulcus as reported previously (e.g., MacAvoy et al. 1991; Tanaka and Fukushima 1998) while the monkeys were rewarded with apple juice for tracking a target spot moving sinusoidally at 0.3 or 0.5 Hz (±10°). The inter-aural midpoint of the animals' heads was positioned close to the axis of horizontal rotation. The target was a laser spot that was back-projected onto the tangent screen 75 cm in front of the animals' eyes (monkey C, Fukushima et al. 2000, 2002a) and a 22-inch computer display (Sony) positioned 60 cm in front of the animals' eyes (monkeys SI and SH; e.g., Kasahara et al. 2006). Once pursuit-responsive single neurons were encountered, smooth pursuit responses were tested in 4 planes (vertical, horizontal and two oblique planes at 45° angles) to determine the preferred direction. For neurons with horizontal pursuit preferred directions (e.g., Fig. 1A1, B1), we tested whole body horizontal rotation. This is because, in previous studies, vestibular-preferred directions during whole body rotation and pursuit-preferred directions were in the same plane for virtually all FEF pursuit neurons tested (Fukushima et al. 2000).

Using sinusoidal whole body rotation in the presence of a target (e.g., Fig. 1A2-3, B2-3), we first classified pursuit neurons either as gaze-velocity neurons or eye/head-velocity neurons in order to examine a possible difference in the time course of discharge modulation. In the VOR cancellation task (Fig. 1A2, B2), the monkeys tracked a target that moved in space with the same amplitude, direction and phase as the chair rotation. This condition required the monkeys to cancel the VOR so that the eyes remained virtually motionless in the orbit and gaze moved with the chair. In the VOR x1 (Fig. 1A3, B3), the target stayed stationary in space during chair rotation and the monkeys were required to fixate the stationary spot by a

perfect (x1) VOR so that gaze remained stationary in space. Based on the previous criteria (Fukushima et al. 2000), we classified pursuit neurons as gaze-velocity (e.g., Fig. 1A1-3), if 1) their peak modulation occurred for eye (pursuit) and head (VOR cancellation) movements in the same direction; 2) and modulation was lower during VOR x1 than during VOR cancellation. The pursuit neurons that responded to whole body rotation but that did not meet the above criteria were classified as eye/head-velocity neurons, because such neurons coded eye velocity during VOR x1 in previous studies (also Fig. 1B3 vs B1). Some of them exhibited biphasic modulation during VOR cancellation (e.g., Fig. 1B2, Fukushima et al. 2000).

Whole body rotation velocity-step (20°/s for 1 s, peak acceleration ~600°/s^2) was, then, applied for each neuron with random inter-trial intervals in the horizontal plane as reported previously (e.g., Tsubuku et al. 2006). Target position was controlled using the horizontal chair position obtained from a potentiometer attached to the turntable. The VOR x1 and VOR cancellation task conditions were tested. For many neurons, we also tested the effects of chair rotation in complete darkness without a target (VORd). Typically 20 trials were tested for each of leftward and rightward whole body rotation. Each of these three task conditions was tested as a block. VOR x1 was tested first, followed by VOR cancellation. VORd was tested last. For comparison, discharge modulation during smooth pursuit was also tested by moving the target in a ramp trajectory at 20°/s (for 1 s).

Eye- and target- position signals were digitized at 500 Hz using a 16-bit A/D board (National Instruments) on a Macintosh Quadra computer. The occurrence of neuronal discharge was detected at 100 kHz clock rate. Signals from eye coils and chair position potentiometer were differentiated by analogue circuits (DC-100 Hz, -12 dB/octave) to obtain velocity. Saccades were marked with a cursor on eye velocity traces and removed using the interactive computer program as described previously (Singh et al. 1981; Fukushima et al. 2000). To examine latency of neuronal discharge in each task condition, we first aligned 20-40 trials on the stimulus onset. Because discharge may have been affected by saccades, we then omitted all traces in which saccades appeared within ~100 ms of the stimulus onset. We superimposed typically over

10 traces with respect to the stimulus onset, and constructed histograms of cell discharge in 2 ms time bins. The control values (mean and standard deviations SD) for eye and cell responses were calculated from the 200 ms interval immediately before the stimulus onset. Onset of the neuronal responses to the onset of stimulus velocity (i.e., target motion onset for smooth-pursuit, chair rotation onset for whole body rotation) was determined as the time at which the mean discharge rate exceeded 2SD of the control value as described previously (e.g., Akao et al. 2005). For neurons that exhibited biphasic modulation during sinusoidal whole body rotation, responses to whole body-step rotation were analyzed for the direction in which stronger modulation was obtained. Latencies of eye movement responses were analyzed as described previously (Fukushima et al. 2001a; Akao et al. 2004).

Recording locations of one of the 3 monkeys (C) were described earlier (Fig. 11B of Fukushima et al. 2002a) and were confirmed to be within the caudal FEF in the fundus of the arcuate sulcus. Two other monkeys are still being used for other experiments.

Figures 1 and 2 near here

Results

Vestibular responses of FEF pursuit neurons to whole body step-rotation

We tested the effects of whole body step-rotation on a total of 29 pursuit neurons in the caudal FEF. By comparing discharge modulation during sinusoidal pursuit and during sinusoidal whole body rotation for VOR cancellation and x1 (e.g., Fig. 1A1-3, B1-3), these neurons were classified either as gaze-velocity neurons (n=15) or eye/head-velocity neurons (n=14) based on the previous criteria (see Methods, Fukushima et al. 2000). Latencies of these neurons were, then, tested using whole body step-rotation. Of the 29, 24 neurons were tested during VOR cancellation (13 gaze-velocity, 11 eye/head-velocity neurons), 26 neurons during VOR x1 (12 gaze-velocity, 14 eye/head-velocity neurons), and 23 neurons during chair rotation in complete darkness without a target (VORd)(13 gaze-velocity, 10 eye/head-velocity neurons). All three vestibular task conditions were tested in 18

pursuit neurons (9 gaze-velocity, 9 eye/head-velocity neurons) using whole body step-rotation.

Representative responses to whole body step-rotation are illustrated in Fig. 1 for 2 neurons (A, gaze-velocity neuron; B, eye/head-velocity neuron) during VOR cancellation (A5, B5), VOR x1 (A6, B6), and during VORd (A7, B7). Both neurons had a leftward preferred direction during smooth pursuit (Fig. 1A1, 4, B1, 4), and following chair rotation (A5, B5, downward arrowheads) they exhibited a burst of spikes at short latencies during whole body rotation (A5-7, B5-7, upward arrows). The gaze-velocity neuron shown in Fig. 1A exhibited clear vestibular responses during whole body rotation towards left (A5-7), whereas the eye/head-velocity neuron (Fig. 1B) discharged clearly during rightward whole body rotation (B5-7). Response latencies induced by chair rotation (Fig. 1A5-7, B5-7, upward arrows) were clearly shorter than the response latencies during pursuit for the same neurons (Fig. 1A4 and B4, upward arrows, e.g., MacAvoy et al. 1991; Tanaka and Fukushima 1998), indicating that the former responses cannot be induced by visual feedback.

Figure 2(A-C) summarizes latency distributions of gaze-velocity neurons and eye/head-velocity neurons examined during VOR x1 (A), VOR cancellation (B), and VORd (C). Latency distributions of the two groups of neurons were similar in each task condition. Furthermore, the latencies of initial responses (i.e., < ~80ms after stimulus onset) to whole body step-rotation of individual neurons were basically similar in the 3 task conditions (Fig. 2A-C). The percentages of neurons that exhibited latencies shorter than 80 ms were 73% (19/26) during VOR x1 (Fig. 2A), 67% (16/24) during VOR cancellation (Fig. 2B), and 70% (16/23) during VORd (Fig. 2C). Only a minority of pursuit neurons tested (6/23=26%, Fig. 2C, NR) did not respond to chair step-rotation during VORd (Fig. 2C). Figure 2D plots all data points that responded. The majority of pursuit neurons tested (~70%) had response latencies to chair rotation shorter than 80 ms and the modal value was 24 ms (Fig. 2D). The shortest latency was 20 ms (Fig. 2D). Initial eye movement responses (i.e., VOR) were similar in all 3 vestibular task conditions tested (e.g., Fig. 1A5-7, B5-7), and their latencies

were 12-16 ms (mean 14 ms).

Figure 2E plots latencies of each neuron to the onset of target motion during smooth pursuit without vestibular stimulation. Latencies ranged from 80 to 240 ms, and the distributions were similar for the two groups of neurons. The modal and mean values for all neurons tested were 95 and 110 ms, respectively. These latencies (Fig. 2E) are clearly longer than vestibular latencies (Fig. 2D). For comparison, latencies of pursuit eye movements were typically 100-110 ms (Fig. 3F).

Figure 3 near here

Interaction of pursuit- and vestibular- signals in the caudal FEF

To illustrate the time course of discharge modulation of the two groups of neurons during different vestibular task conditions, Fig. 3 summarizes population responses of a total of 18 pursuit neurons. Gaze-velocity neurons (n=9) are shown in Fig. 3A and eye/head-velocity neurons (n=9) are shown in Fig. 3B during VOR x1 (blue), VOR cancellation (red), VORd (black), and smooth pursuit (green) in which all task conditions were tested for the same neurons. The discharge rate clearly increased at 25-30 ms after the onset of chair rotation during the three vestibular task conditions, and these initial responses were basically similar between gaze-velocity neurons and eye/head-velocity neurons (Fig. 3A, B). The discharge rate for smooth pursuit increased at 80-90 ms after the onset of target motion. At $\geq$100 ms following the onset of chair rotation, gaze-velocity neurons exhibited higher discharge rate during VOR cancellation (Fig. 3A, red) compared to the discharge rate during VOR x1 and VORd (Fig. 3A, blue and black), whereas eye/head-velocity neurons tended to show higher discharge rate during VOR x1 (Fig. 3B, blue) compared to the discharge rate during VOR cancellation and VORd (Fig. 3B, red and black).

To illustrate the difference in discharge modulation between VOR cancellation and x1 for the two groups or neurons, we subtracted mean discharge rate during VOR x1 from mean discharge rate

during VOR cancellation. These differences are plotted in Fig. 3C for the two groups of neurons (thick and thin lines for gaze-velocity and eye/head-velocity neurons, respectively). The mean discharge rate difference (±SD) during 200 ms before stimulus onset was similar between the two groups of neurons and was 4±5 spikes/s, clearly deviated from zero. The mean discharge rate during the initial vestibular responses (i.e., <80 ms following the onset of chair rotation) was similar to the control value, and there was no significant difference in the mean difference between the two groups of neurons. However, at ≥100-110 ms following the onset of chair rotation, the difference significantly increased for gaze-velocity neurons, whereas the difference significantly decreased for eye/head-velocity neurons (Fig. 3C). The difference in discharge rate between the two groups of neurons diverged at ~90 ms following the onset of chair rotation (Fig. 3C, arrow).

These differences in discharge modulation between the two groups of neurons (Fig. 3C) were most probably induced by discharge modulation associated with smooth pursuit, because by subtracting discharge rate during VOR x1 from discharge rate during VOR cancellation, vestibular components should have been nullified and the main remaining components should have been pursuit-related modulation during the two vestibular task conditions. To test this possibility, we compared the discharge rate difference and discharge modulation during pursuit for the two groups of neurons. We tested whether the time course of discharge rate difference was predicted by the discharge modulation associated with smooth pursuit (Fig. 3A, B, green) by the following equation (Gomi et al. 1998; Kurkin et al. 2003):

$$f(t) = G*\text{smooth pursuit}(t-t0) + A \qquad (1)$$

where f (t) is reconstructed discharge rate at time t; G is sensitivity of modulation associated with smooth pursuit, t0 is time shift between pursuit response and the discharge rate difference seen in Fig. 3C; A is a bias term combining resting discharge rate and DC shift. The results are summarized in Fig. 3D and E for population response of gaze-velocity neurons (D) and eye/head-velocity

neurons (E). The discharge rate difference of gaze-velocity neurons between VOR cancellation and x1 (Fig. 3D) was well predicted by pursuit modulation with G = 0.45, t0 = 16 ms, and A = 0. Similarly, the discharge rate difference of eye/head-velocity neurons between VOR cancellation and x1 (Fig. 3E) was predicted by pursuit modulation with G = -0.33, t0 = 0 ms, and A = 6 spikes/s. Furthermore, the latencies of response divergence of the two groups of neurons are consistent with the latencies of discharge modulation associated with smooth pursuit (Fig. 3D, E, arrows).

For comparison, Fig. 3F plots de-saccaded mean eye velocity during the 4 task conditions. Divergence of eye velocity between VOR cancellation and x1 occurred at 115 ms (Fig. 3F, arrow), consistent with the appearance of smooth pursuit (see Discussion).

Discussion

Vestibular signals in the caudal FEF

Our results extend previous findings (Fukushima et al. 2000; Ebata et al. 2004) by showing that the majority of tested pursuit neurons in the caudal FEF responded to whole body step-rotation at short latencies (20-24 ms). Because the latencies of these initial responses were basically similar not only in the three vestibular task conditions but also between gaze-velocity neurons and eye/head-velocity neurons (Figs. 1, 2, 3A, B), we interpret these to be vestibular responses induced by semicircular canal inputs.

Anatomical studies in rats have reported that vestibulo-thalamic fibers that originate from the superior vestibular nucleus and rostral-to-middle parts of the medial vestibular nucleus project to the lateral part of the thalamic parafascicular nucleus (corresponding to the centromedian nucleus in primates), the transitional zone between the ventrolateral nucleus (VL) and the ventral posterolateral nucleus, the lateral part of the centrolateral nucleus, and the dorsal part of the caudal VL. These thalamic areas project to the frontal cortex including FEF (Shiroyama et al. 1999). Ebata et al. (2004), using the laminar field potential analysis, suggested that vestibular-evoked potentials

in the caudal FEF of monkeys were directly conveyed to the cortex through the thalamus, consistent with the anatomical studies (Shiroyama et al. 1999).

We think that the earliest vestibular responses in the present study (Fig. 2D) were induced by the trisynaptic pathway from the labyrinth through the vestibular nuclei and the thalamic regions (Fukushima 1997 for a review) for the following reasons. Ebata et al. (2004) reported vestibular evoked, presumably trisynaptic, potentials in the fundus of the arcuate sulcus in anesthetized monkeys following electrical stimulation of the labyrinth at latencies of 4.8-6.3 ms but with peak responses at 15 ms (Fig. 1 of Ebata et al. 2004). Because they used electrical stimulation, the time needed for hair cell transduction at the labyrinth is unknown but should not be more than 2 ms. Lisberger et al. (1994) reported that second-order vestibular nuclear neurons of rhesus monkeys discharge at the median latency of 7 ms to horizontal chair step-rotation of 30°/s. Because second-order vestibular neurons discharge typically at a latency of 1 ms following electrical stimulation of the labyrinth (see Wilson and Melvill Jones 1979 for a review), we estimate the time for the major vestibular discharge to reach FEF pursuit neurons following chair step-rotation to be (15+7-1=) 21 ms. This value is close to the modal value (24 ms, Fig. 2D) of our FEF pursuit neurons following chair step-rotation (shortest latency at 20 ms). It should be noted that our vestibular apparatus produced peak acceleration of only ~600°/s^2. Higher acceleration may have further shortened the response latencies. Nevertheless, our results are consistent with the suggestion that the vestibular signals in the majority of pursuit neurons in the caudal FEF have a direct vestibular origin (Ebata et al. 2004).

The exact time needed for FEF neurons to initiate pursuit eye movements is unknown. However, it has been reported that electrical stimulation of the caudal FEF induces smooth pursuit with a latency of ~20 ms (Tanaka and Lisberger 2002; also Gottlieb et al. 1993) and that the majority of FEF pursuit neurons discharge before the initiation of pursuit with a median lead time of 19 to 12

ms (Gottlieb et al. 1994; Tanaka and Fukushima 1998), consistent with the present results (Figs. 2E, 3F). These results suggest that, using vestibular signals, the caudal FEF could participate in initial pursuit eye movements with latencies as short as *40* ms after the onset of whole body step-rotation.

Our results also show that a minority of FEF pursuit neurons (6/23=26%, Fig. 2C, NR) did not respond to chair step-rotation during VORd, although these neurons responded during VOR x1 and cancellation with latencies > 90 ms (Fig. 2A, B). We think that these late responses in the presence of a target were most probably induced by visual feedback and/or pursuit signals. The results of detailed analysis of the time course of discharge modulation of the two groups of FEF pursuit neurons during VOR cancellation and x1 are consistent with this interpretation (Fig. 3D, E, also see below).

Interaction of pursuit- and vestibular- signals in the caudal FEF: gaze-velocity and eye/head-velocity signals

It should be noted that the mean (±SD) discharge rate difference of pursuit neurons between VOR cancellation and x1 before stimulus onset (4±5 spikes/s, Fig. 3C-E) was clearly deviated from zero. The exact reason for this deviation is unknown. However, because each task was tested as a block (see Methods), it may well be that this deviation was related to the monkeys' "set" for VOR cancellation. Set related activity is well known in the motor cortex and premotor and supplementary motor areas (e.g., Shima and Tanji 1993). King et al. (1981) demonstrated that the VOR cancellation task considerably increased a DC rate of a burst-tonic neuron in the interstitial nucleus of Cajal compared to its discharge during the VOR x1 task (see Fig. 3 of King et al. 1981).

The present results also indicate that discharge of the two groups of neurons diverged at ~90 ms following the onset of chair rotation, consistent with the latencies associated with smooth pursuit (Fig. 3C). Moreover, the time course of discharge rate difference was predicted by the discharge

modulation associated with smooth pursuit (Fig. 3D, E). These results suggest that both groups of neurons are involved in the interaction of pursuit- and vestibular signals (see also below).

Cullen et al. (1993) reported pursuit neurons in the brainstem that exhibit gaze-velocity responses during pursuit-vestibular interactions but that do not receive vestibular inputs. These authors have shown that these brainstem-pursuit neurons contribute to VOR cancellation, and suggested that these neurons might receive inputs from gaze-velocity Purkinje cells in the cerebellar flocculus (Cullen et al. 1993). The caudal FEF also contains many gaze-velocity neurons (Fukushima et al. 2000, also in this study). Gaze-velocity signals are also found in the dorsal vermis (Shinmei et al. 2002), the dorsolateral and dorsomedial pontine nuclei, and the nucleus reticularis tegmenti pontis (NRTP, Ono et al. 2004). Because the FEF sends projections to these pontine areas, it could send gaze-velocity signals to the floccular region and dorsal vermis through these pontine nuclei and NRTP (Ono et al. 2004). Chemical inactivation of the caudal FEF impairs not only smooth pursuit but also VOR cancellation (Fukushima et al. 1999a). These results taken together suggest that part of VOR cancellation signals to brainstem pursuit neurons could come from the caudal FEF through gaze-velocity Purkinje cells in the cerebellar floccular region and dorsal vermis (Fukushima 2003).

Not only gaze-velocity signals but also eye/head-velocity signals are represented in the caudal FEF, pontine nuclei, NRTP (Ono et al. 2004), the cerebellar floccular region, and dorsal vermis (Lisberger and Fuchs 1978; Miles et al. 1980; Sato and Noda 1992; Fukushima et al. 1999b; Belton and McCrea 2000; Shinmei et al. 2002). Gaze-velocity signals alone would be insufficient for adequate control of smooth eye movements to a stationary spot in space during whole body rotation (i.e., VOR x1), because virtually no gaze movement would be required in such a situation and gaze velocity neurons become less active (e.g., Lisberger and Fuchs 1978). Although the VOR is a powerful reflex, its gain is <1, so it alone is not perfect to maintain a target image on the fovea

during target- and/or head- movement in primates. In the presence of the visual target stationary in space, the VOR becomes perfect (i.e., VOR x1). Eye/head-velocity neurons are more active in such a situation (Fig. 3B, C, E)(also Fukushima et al. 1999b, 2000; Belton and McCrea 2000; Shinmei et al. 2002). These results taken together suggest that both gaze-velocity and eye/head-velocity neurons are necessary in primates for integration of vestibular inputs and pursuit signals so that the precise control of smooth eye movements can be made to maintain target image on the fovea during pursuit and/or whole body rotation. The close correlation between response divergence of FEF pursuit neurons and eye velocity divergence during VOR cancellation and x1 (Fig. 3C, F) with the latency shift of 25 ms (i.e., 90 ms vs 115 ms) is consistent with this interpretation because the estimated time delay needed for FEF neurons to initiate pursuit eye movements is about 20 ms as discussed above.

Possible involvement of caudal FEF in modulation of vestibular signals during pursuit-vestibular interaction in adaptive behavioral conditions

As discussed above, the present results suggest that, using vestibular signals induced by whole body-step rotation, the caudal FEF could participate in initial pursuit eye movements with latencies as short as *40 ms*. Such short latency pursuit eye movements cannot be achieved if both target motion and chair rotation were applied simultaneously during VOR cancellation or x1 in the present study (Fig. 3D, E), because the initiation of smooth pursuit to *visual* target motion requires at least *100* ms (Fig. 3A, B, F); almost half of this latency is used for visual processing in the retina (see Leigh and Zee 1999 for a review). However, short latency pursuit eye movements may be achieved during a special behavioral condition that could shorten this visual delay. For example, using an estimate of target velocity, pursuit latency could be considerably shortened (e.g., Barnes).

Cross-axis vestibular-pursuit training is an example that we have used to understand the role of vestibular signals in adaptive pursuit eye movements by applying spot motion in the plane

orthogonal to the plane of whole body rotation (Fukushima et al. 1996, 2001a, b; Sato et al. 1999; Tsubuku et al. 2006; also Walker and Zee 2002). Normally, VOR appears only in the plane specific to the rotation plane. Cross axis paradigms have the advantage that they can detect qualitatively different eye movement responses (which normally does not exist) from the normal VOR and allows us to examine such components separately from the collinear VOR. This is because target motion is not applied along the rotation plane such as the VOR cancellation task. We have shown that cross-axis vestibular-pursuit training induces adaptive smooth eye movements in head-stabilized monkeys. If the target motion is synchronized with chair rotation (but in the orthogonal plane), latencies of initial pursuit eye movements to spot motion during step-rotation shortened adaptively from about 100 ms (i.e., normal pursuit latency) to 42 ms (latencies too short for visual feedback, Fukushima et al. 2001a). Vergence-vestibular interaction training also shortened the latencies of initial smooth eye movements to target motion-in-depth during step-rotation from 160-180 ms (i.e., normal vergence latency) to a mean latency as short as 53 ms (Akao et al. 2004; also Sato et al. 2004).

The neural substrates for cross-axis pursuit eye movements are still unknown. However, we have suggested that the caudal FEF could participate in adaptive pursuit eye movements (Fukushima et al. 2005 for a review), because the majority of pursuit neurons there not only respond to whole body rotation, but also respond to smooth pursuit in fronto-parallel planes and pursuit-in-depth (Fukushima et al. 2000, 2002b; Akao et al. 2005). In preliminary studies, we recorded several FEF pursuit neurons during cross-axis vestibular-pursuit training. All of tested neurons exhibited responses associated with adaptive smooth eye movement responses (Fukushima et al. 2005). The present results support our suggestion, because, as discussed above, the majority of our FEF pursuit neurons receive vestibular signals early enough for them to contribute to the vestibularly induced-short latency pursuit eye movements (i.e., < 42-53ms).

It should be noted that such short latency responses of FEF pursuit neurons become robust after training (see Fig. 4 of Fukushima et al. 2005). The monkeys in the present study were not subjected to cross-axis vestibular-pursuit training, and FEF pursuit neurons exhibited initial responses that were basically similar in the different vestibular task conditions. It is conceivable that further vestibular-pursuit training would result in modulation of the initial vestibular responses carried by FEF pursuit neurons (see Fig. 4A and C of Fukushima et al. 2005) so that these signals could effectively be used for initiation of subsequent, task-dependent, pursuit eye movements (e.g., Tsubuku et al. 2006). This possibility should be tested in future studies.

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Legends for Figures

Fig. 1. Pursuit neuron response to whole body rotation. A and B show responses of a gaze-velocity neuron (A) and eye/head-velocity neuron (B) during sinusoidal (A1, B1) and ramp (A4, B4) target motion. A2-3 and B2-3 are responses during sinuosoidal whole body rotation when the target moved together with the whole body (VOR cancellation, A2, B2), and when the target remained stationary in space during rotation (VORx1, A3, B3). A5-7 and B5-7 are responses during whole body rotation-step during VOR cancellation (A5, B5), VOR x1 (A6, B6), and chair rotation-step in darkness without a target (VORd, A7, B7). Saccades were deleted in eye velocity traces in A1-3 and B1-3. In all other traces, saccade velocities are clipped to indicate the occurrence of saccades. In A4-7 and B4-7, we deleted traces in which saccades appeared within 100 ms of the stimulus onset. Vel and pos are eye velocity and position, respectively. HE and HE vel indicate horizontal eye position and horizontal eye velocity, respectively. Time scales are identical in A4-7 and B4-7. These neurons were recorded in the left caudal FEF. In all traces, upward and downward directions are rightward and leftward, respectively. Downward open arrowheads in A4-5 and B4-5 indicate stimulus onset. Onset and direction of chair rotation that is indicated in A5 are identical in A6 and A7. Similarly, onset and direction of chair rotation that is indicated in B5 are identical in B6-7. Upward arrows in A4-7 and B4-7 indicate onset of cell responses.

Fig. 2. Latency histograms of discharge of gaze-velocity and eye/head-velocity neurons in the caudal FEF to whole body rotation-step and ramp target motion. The two groups of neurons are indicated by the keys. A, during VOR x1. B, during VOR cancellation. C, during chair rotation in complete darkness without a target (VORd). NR in C indicates no response. All responding points in A-C are combined in D. E, latency distribution of discharge of the two groups of neurons during smooth pursuit.

Fig. 3. Population responses of gaze-velocity and eye/head-velocity neurons in the caudal FEF during chair rotation-step and ramp target motion and eye velocity responses. Averaged discharge rates of 9 gaze-velocity neurons (A) and 9 eye/head-velocity neurons (B) are shown during VOR x1, VOR cancellation, and chair rotation in complete darkness without a target (VORd) and smooth pursuit as indicated by the keys. C plots difference in mean discharge rate between VOR cancellation and x1 for gaze-velocity neurons (thick line) and eye/head-velocity neurons (thin line). Arrow indicates the divergent point between the two groups of neurons. D plots the discharge rate difference between VOR cancellation and x1 for gaze-velocity neurons (red) and prediction of smooth pursuit-related discharge modulation of gaze-velocity neurons (green) using the equation (1). E plots the discharge rate difference between VOR cancellation and x1 for eye/head-velocity neurons (blue) and prediction of smooth pursuit-related discharge modulation of eye/head-velocity neurons (green) using the equation (1). All discharge histograms were constructed with 4 ms time bins. F plots de-saccaded eye velocity during the 4 task conditions as indicated by the keys. Eye velocities while recording the two groups of neurons were similar, and therefore, all traces were averaged for each of the 4 task conditions. Eye velocity directions were corrected as positive for all traces. Arrow indicates the divergent point for eye velocity between VOR cancellation and x1.

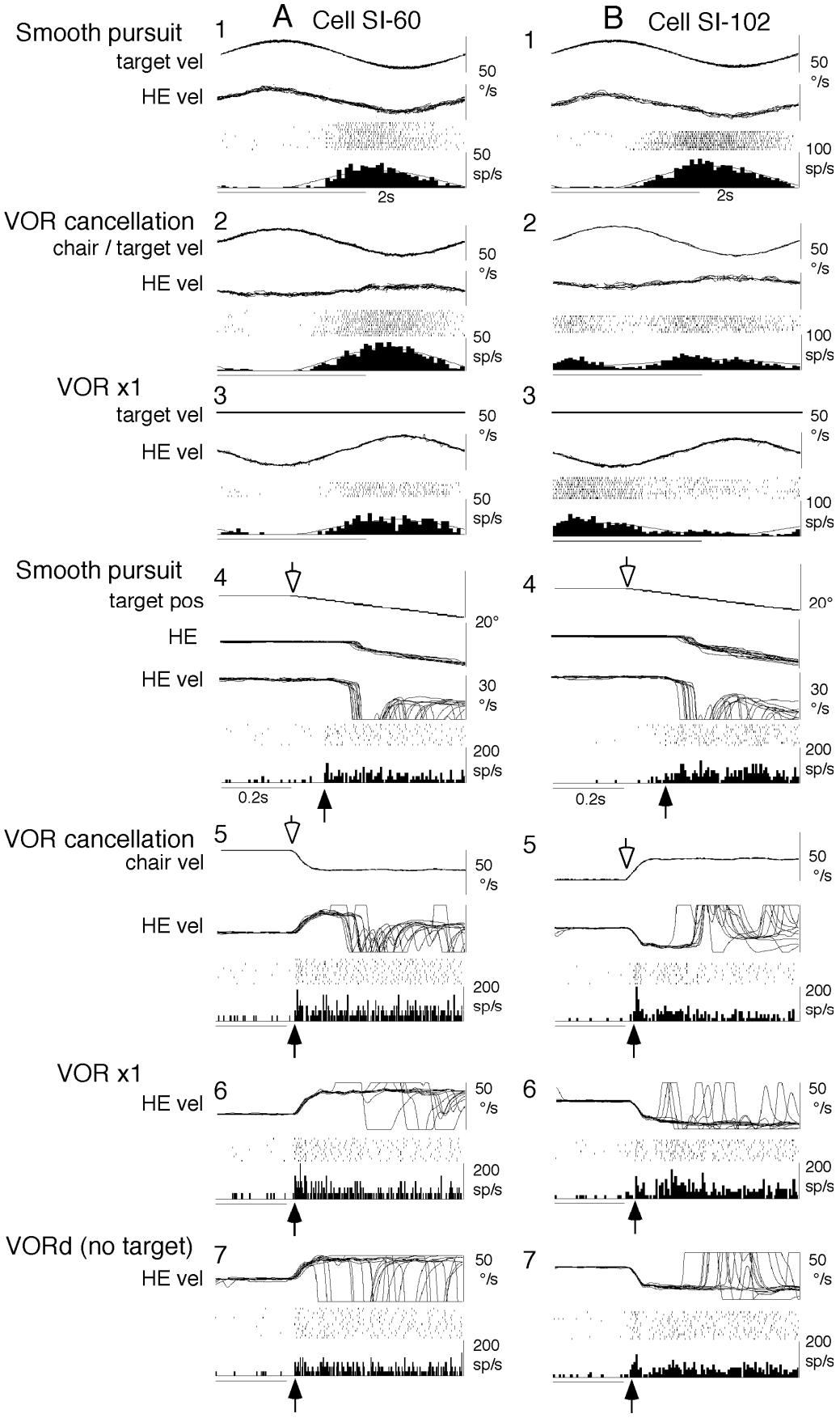
A Cell SI-60
B Cell SI-102
Smooth pursuit
target vel
HE vel
VOR cancellation
chair / target vel
VOR x1
target pos
HE
VORd (no target)
50 °/s
100 sp/s
50 sp/s
200 sp/s
30 °/s
20°
2s
0.2s
1
2
3
4
5
6
7

Fig. 1

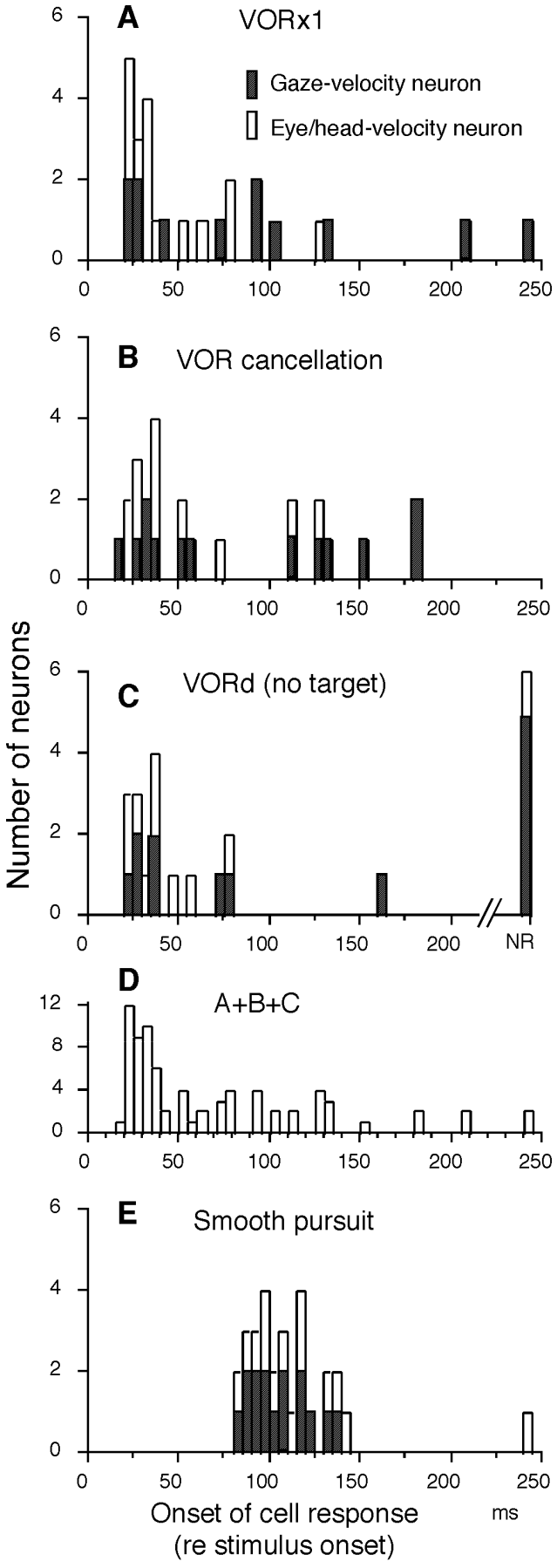
A
VORx1
Gaze-velocity neuron
Eye/head-velocity neuron
B
VOR cancellation
C
VORd (no target)
NR
D
A+B+C
E
Smooth pursuit
Number of neurons
Onset of cell response
(re stimulus onset)
ms

Fig. 2

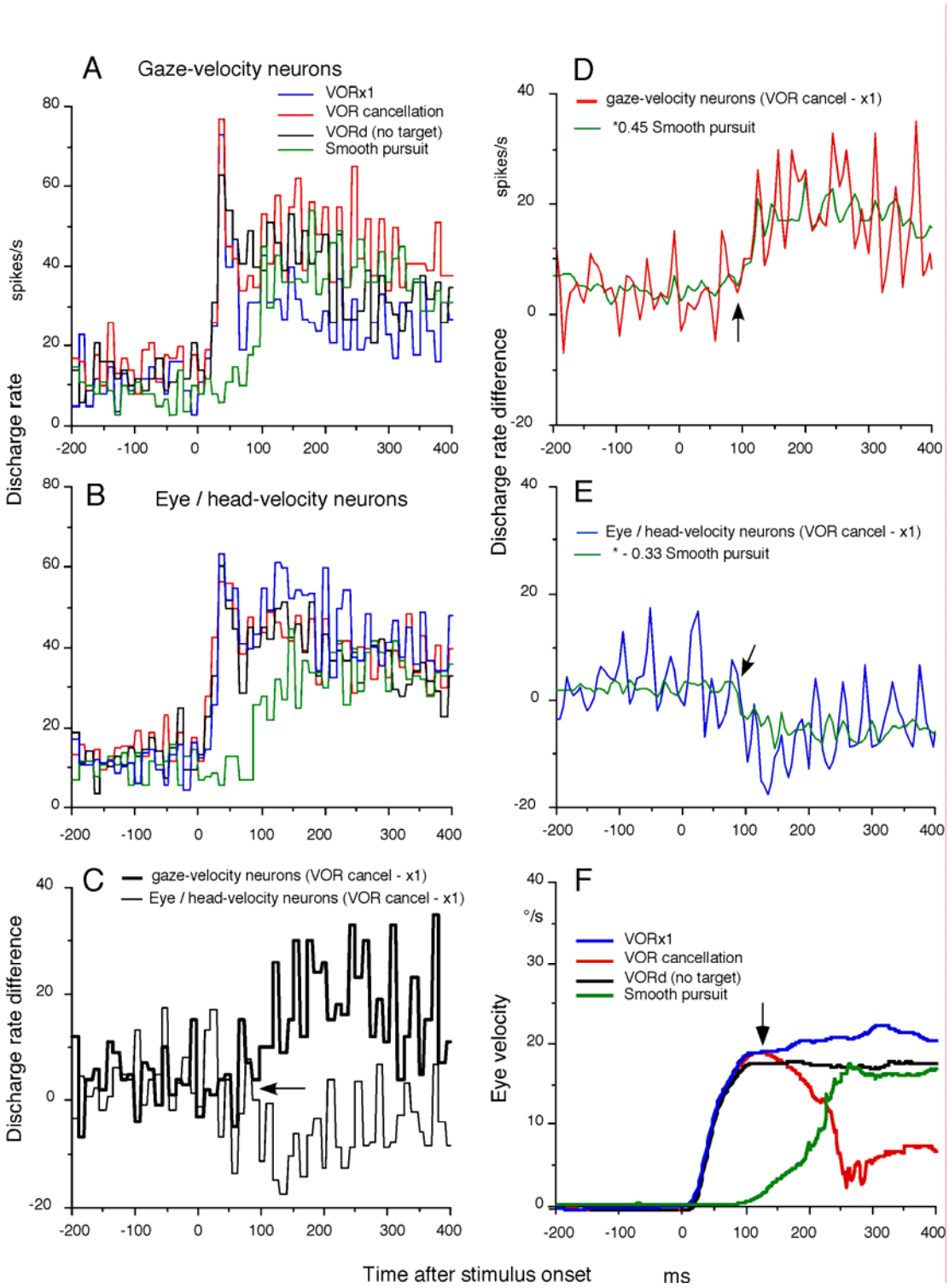
A
Gaze-velocity neurons
VORx1
VOR cancellation
VORd (no target)
Smooth pursuit
spikes/s
Discharge rate
B
Eye / head-velocity neurons
C
gaze-velocity neurons (VOR cancel - x1)
Eye / head-velocity neurons (VOR cancel - x1)
Discharge rate difference
D
gaze-velocity neurons (VOR cancel - x1)
*0.45 Smooth pursuit
spikes/s
Discharge rate difference
E
Eye / head-velocity neurons (VOR cancel - x1)
* - 0.33 Smooth pursuit
F
°/s
VORx1
VOR cancellation
VORd (no target)
Smooth pursuit
Eye velocity
Time after stimulus onset
ms

Fig. 3