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MOSSY FIBRES SENDING RETINAL-SLIP, EYE, AND HEAD VELOCITY SIGNALS TO THE FLOCCULUS OF THE MONKEY

BY HIROHARU NODA

From the Department of Visual Sciences, School of Optometry, Indiana University, Bloomington, IN 47405, U.S.A.

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SUMMARY

1. Discharges of mossy fibres were recorded from the cerebellar flocculus of monkeys trained to fixate a small visual target and to track the target when it moved slowly. The experimental paradigms used were designed to study neural responses to retinal-slip velocity, eye velocity, or head velocity, individually or in combination.

2. Among 485 mossy-fibre units recorded from the flocculus, sixty-four units (or 13%) responded to movement of the visual stimulus in the horizontal plane.

3. Two distinct groups of visual mossy fibres were found: they were designated 'visual units' (thirty-nine/sixty-four units or 61%) and 'visuomotor units' (twenty-five/sixty-four units or 39%).

4. The visual units responded exclusively to the retinal-slip velocity. Stationary fixation was necessary for clear cyclic modulation of activity. Their responses declined when the retinal-slip velocity was reduced by eye movements in the same direction.

5. The responses of the visual units were directionally selective and lagged behind the occurrence of 'turnabouts' (changes in direction of stimulus movement) and their peak discharges also lagged the occurrence of peak velocity.

6. Each visual unit had a limited range of velocity sensitivity; in some units the range covered the velocity range of smooth-pursuit eye movements.

7. The visuomotor units had visual receptive fields in the peripheral retina (outside of the central 10 deg); they received also oculomotor and vestibular signals.

8. When the head was stationary, the visuomotor units responded to the target velocity (or visual stimulus velocity) which is the algebraic sum of the retinal-slip velocity and the eye velocity. Their responses reflected the retinal-slip velocity during stationary fixation and the eye velocity during smooth-pursuit eye movements. The responses to stimulus movements were, therefore, almost identical regardless of whether the eyes remained stationary or moved with the stimulus.

9. In response to sinusoidal stimulus movements, the responses of the visuomotor units frequently preceded the stimulus velocity, and the phase lead relative to the velocity curve increased when the frequency of sinusoidal movements was increased. This reflected a relatively constant lead of neural discharges (*circa* 125 ms) during various frequencies.

10. When the head was moved, the responses of the visuomotor units were

dominated by the head velocity, and discharges in response either to the retinal-slip velocity or to the eye velocity (both in the direction opposite to the head velocity) were occluded.

11. The response characteristics of the visuomotor units such as (1) representing the target velocity regardless of eye movements (in which two velocity signals must be taken into account), (2) the phase lead of the responses relative to the stimulus velocity, and (3) the switching mechanism (where the head-velocity signal dominates the response), suggest that these fibres signal more highly integrated information than simple sensory neurones and that information concerning object movement (if the head is stationary) is produced by their parent brain-stem neurones. The flocculus, therefore, receives a highly processed input in addition to the already described input signals concerning retinal-slip velocity, eye velocity, and head velocity via independent mossy-fibre channels.

INTRODUCTION

The maintenance of images on the fovea, a prerequisite for visual function, is the responsibility of the pursuit eye movement system. Since a smooth-pursuit eye movement is controlled to match the angular velocity of the eye to the velocity of the target (head stationary), information concerning the target velocity must be available to the system. In situations where the head and/or eyes are allowed to move, however, information about the target velocity is not directly available from the visual system. The target velocity, therefore, must be computed in the brain based on information concerning the relative motions of (1) target and eye (retinal-slip velocity), (2) eye and head (eye velocity), and (3) head and ground (head velocity) (Young, 1971).

Recent single-unit studies on trained monkeys have agreed that the flocculus is part of the neural pathways underlying visually controlled smooth-pursuit eye movement (Miles & Fuller, 1975; Lisberger & Fuchs, 1978*a*; Noda & Suzuki, 1979*b*; Miles, Fuller, Braitman & Dow, 1980). The signals corresponding to the eye- and head-velocity signals have been discovered in the inputs to the flocculus by recording mossy-fibre activity (Lisberger & Fuchs, 1978*b*; Miles *et al.* 1980). In fact, when tested with a predictable target motion, well-trained monkeys can execute almost perfect smooth-pursuit eye movements. The retinal-slip velocity becomes thereby theoretically negligible because the eye velocity matches perfectly the target velocity. In such cases, the information about retinal-slip velocity does not play a significant role in the modification of output signals by the pursuit system. For this reason, the role of visual input has not been emphasized in the models of Lisberger & Fuchs (1978*a*) or of Miles and his co-workers (Miles *et al.* 1980).

Practically, however, visually-guided smooth pursuit is frequently accompanied by retinal slip. This becomes particularly obvious when the target motion is unpredictable. In order to match the velocity of the tracking eye movement to the target velocity in such eye movements, information about the retinal slip becomes absolutely necessary. To qualify as part of the smooth-pursuit control system, therefore, the flocculus must receive information about the retinal slip. The existence of visual inputs to the flocculus of the monkey has been reported (Miles & Fuller, 1975; Waespe,

Büttner & Henn, 1981). However, partially because the fibres carrying visual information comprise only a small percentage of the afferent fibres, the properties of visual-input signals to the primate flocculus are practically unknown. The aim of the present investigation was to study the characteristics of visual-input signals that are brought to the flocculus of the monkey. In addition to the visual mossy fibres whose response characteristics are already familiar among the neurones in the brain stem, we discovered a group of mossy fibres which receive converging visual, oculomotor, and vestibular inputs and provide the flocculus with highly integrated information about the absolute target velocity. A preliminary report on some of the purely visual mossy fibres has been presented elsewhere (Noda, 1981).

METHODS

Nine pig-tailed macaques (*Macaca nemestrina*) were used in the present study. Following an initial training period, each monkey was intubated under Ketalar sedation and was deeply anaesthetized with a gas mixture of 50% nitrous oxide and oxygen plus a varying amount of methoxyflurane. A search coil was implanted on one eye using the method described by Judge, Richmond & Chu (1980). A bone adaptor (Trent–Wells Inc.) was implanted over the occipital lobe for later insertion of micro-electrodes. It was placed in a trephine hole so that when a hydraulic microdrive was mounted, the micro-electrode was directed to a point on the Horsley–Frankfurt stereotaxic zero and 10 mm lateral from the mid line. For stabilizing the head during recordings, two transverse tubes were placed on the skull and embedded in dental acrylic cement.

Each animal was used for daily experiments over a period of 6–10 months following an initial training period. During this period the monkey learned (1) to come out of a cage and enter a primate chair voluntarily (usually for a fruit-juice reward), (2) to sit quietly in the chair for 2–3 h, and (3) to learn that lever pressing is related to the reward. Most monkeys learned these tasks within 2 weeks and they appeared to be comfortable in the chair for several hours. Some monkeys did not become completely comfortable with these tasks. However, we were usually unsuccessful in teaching these animals more advanced behavioural tasks and, therefore, they were not used for the present experiments.

The monkeys which passed the initial screening were trained to fixate a small spot of red light from a neon laser. A juice reward was contingent upon the release of a lever during a brief presentation of a green spot following the red-spot period. By this procedure, the monkeys were trained to fixate on the red spot and to maintain fixation even if the position of the spot was changed. The calibration of the magnetic search-coil potentials in relation to eye position could be obtained by eliciting sinusoidal tracking eye movements in either the horizontal or the vertical plane.

During an experiment, a monkey was seated in the chair. By two pairs of bars inserted into two transverse tubes on the skull, the head of the monkey was affixed to a frame that provided support for the chair. This system completely immobilized the head without application of painful pressure. The animal was placed in a small room facing a window that had been furnished with a rear-projection screen. When the animal was placed 57 cm from it, the screen subtended 110 deg of visual angle horizontally and 90 deg vertically. A background random dot pattern (interspot luminance: 0·035 ft.lm) was projected on and filled the tangent screen. When the visual pattern was not projected, the small room was completely dark. Typically, the visual stimulus was moved sinusoidally in the horizontal plane between 10 deg right and 10 deg left at frequencies from 0·2 Hz to 0·7 Hz.

In order to study the size and location of the visual receptive field, a random dot pattern (consisting of dark spots of irregular shapes and various sizes) was moved sinusoidally in various parts of the tangent screen. For this purpose, the presentation of the moving pattern was limited either to the central 10 deg of fixation (by projecting the pattern through a circular window) or to the area outside the central 10 deg (by covering the screen with a circular disk), while the animal fixated on the red spot (the spot in a circle in Fig. 8). The animal was then able to see the moving pattern only in the dotted area shown in Figs. 8 and 9*C*.

Vestibular stimulation was applied by rotating the primate chair sinusoidally about the vertical axis of the body while the animal fixated on an l.e.d. (light-emitting diode) spot which was mounted on a bracket extending from the chair and which therefore moved with the animal. Thus the eye movements caused by the vestibulo-ocular reflex (v.o.r.) could be suppressed (the paradigm is illustrated in Fig. 9*B*).

For recording mossy-fibre activity, we used sharp electrodes (made of Elgiloy orthodontic stainless-steel wires and coated with Isonel 31) which were 2–3 μm in diameter at about 5 μm from the tip. Their resistance ranged from 5–15 MΩ. Extracellular action potentials were led through an FET pre-amplifier (Dagan 2400) with a bandpass of 35–10 KHz and were displayed on an oscilloscope. The data were continuously monitored on a polygraph after transformation of the action potentials into recordable pulses through a Schmitt trigger. When the discharges were related to visual-stimulus movements, the data (original spikes, pulses from the Schmitt trigger, eye and head positions, stimulus markers indicating various stimulus conditions) were recorded on magnetic tapes for later detailed analysis, using a fourteen-channel magnetic tape-recorder (Ampex PR2200). Statistical computations were performed by a PDP 11/23 computer.

Data were analysed by constructing phase histogram (Figs. 3, 4, 6, 7, 8 and 9). The phase histogram evaluated the probability of neural firing with respect to the phase of the stimulus. It was constructed usually from ten responses selected from twelve to fifteen stimulus cycles, based on the stability of the gaze on the target. A bin width of either 20 or 40 ms was found to be appropriate for most responses. A running average of five (seven or nine) consecutive bins was computed and the number of bins which yielded the most effective smoothing without significant distortion of the response was found by trial and error. For the instantaneous discharge rate shown in Fig. 5, we replaced each impulse by a Gaussian function (width = 25 ms) as originally described by MacPherson & Aldridge (1979).

Representative recording sites were marked with an iron deposit. A potential of 3 V was applied across the steel micro-electrode for 2–3 s. When the iron was reacted with potassium ferrocyanide, it was found to result in a characteristic blue reaction spot of approximately 150 μm diameter in the Nissl-stain background.

RESULTS

Among 485 mossy-fibre units recorded from the flocculus, sixty-four units (or 13 %) responded to movements of the visual stimulus. The others discharged in bursts associated with saccadic eye movements. In the latter, the bursts with saccades persisted even in complete darkness, indicating that they represented oculomotor signals and did not reflect quick retinal slip accompanying the saccades. Details of their discharges in relation to saccades have already been described (Noda & Suzuki, 1979*c*). A total of 179 mossy-fibre units were also tested during sinusoidal rotation of the primate chair in complete darkness; forty-three units (or 24 %) responded. The visually responsive mossy-fibre units were intermingled with these saccade-related and/or vestibular mossy-fibre units. As seen in Fig. 1, the histologically identified recording sites of twenty-two visual units tended to aggregate in the peduncle of the anterior flocculus (folia 5–10).

Identification of mossy-fibre units

The characteristic discharge patterns of various floccular neurones during eye movements (Noda & Suzuki, 1979*a*, *b*, *c*; Noda & Warabi, 1982) helped us to identify the position of an electrode during the experiment. The identification of the layers facilitated the separation of one kind of unit from another.

Fig. 2*A* and *B* shows a typical spike configuration from a cellular unit (*A*) and an axonal unit (*B*). Among many criteria which have been conventionally used, the longer spike duration and the slower rise of the positive phase were used to distinguish

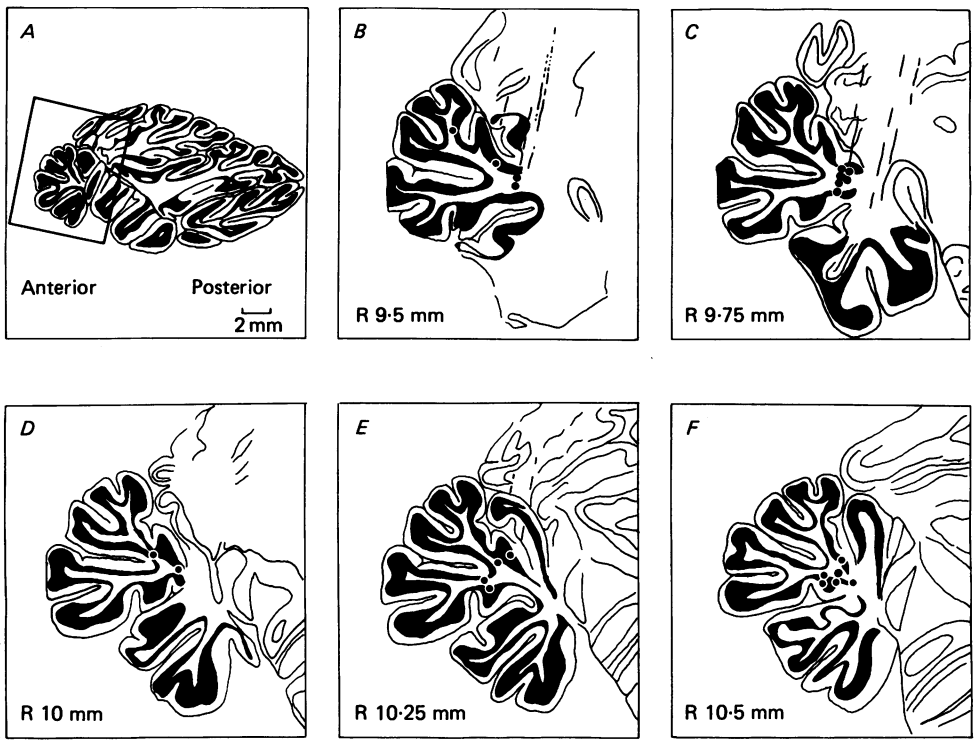

Fig. 1. Histological locations of mossy-fibre units (●) identified by iron deposit in five monkeys. The recording sites which were found in the left flocculus have been projected to the sections showing identical lobus configurations in the right flocculus. *A*, a parasagittal section of the right cerebellum, orienting sections *B* to *F*. R, right.

a cellular unit from an axonal unit, which showed a shorter spike duration and a steeper rise. Complex spikes from the Purkinje-cell activity (marked with a circle in Fig. 2*C*) helped us to identify the Purkinje-cell layer. When the tip of the electrode was in the molecular layer, either complex spikes alone or these intermingled within simple spikes were recordable. When the electrode approached a Purkinje cell from the molecular layer, the amplitude of complex spikes decreased as that of simple spikes increased. The distinction between the granular layer and white matter was difficult to make electrophysiologically unless granular cell activity was identifiable. Both structures were, however, characterized by vigorous saccade-related swish in the background activity.

Three kinds of axonal units are recordable in the white matter: mossy and climbing fibres and the axons of Purkinje cells. Fig. 2 shows typical examples of a mossy-fibre unit (*D*), a climbing-fibre unit (*E*), and axon spikes from a Purkinje cell (*F*) during sinusoidal movement of a visual pattern. Mossy-fibre units were identified by eliminating climbing-fibre and Purkinje-cell units. Because of the low-frequency discharges that corresponded to the appearance of the complex-spike discharges of Purkinje cells (see *C*), climbing-fibre units could be easily identified (*E*) and were eliminated. Discharges of floccular Purkinje cells during sinusoidal movements of a

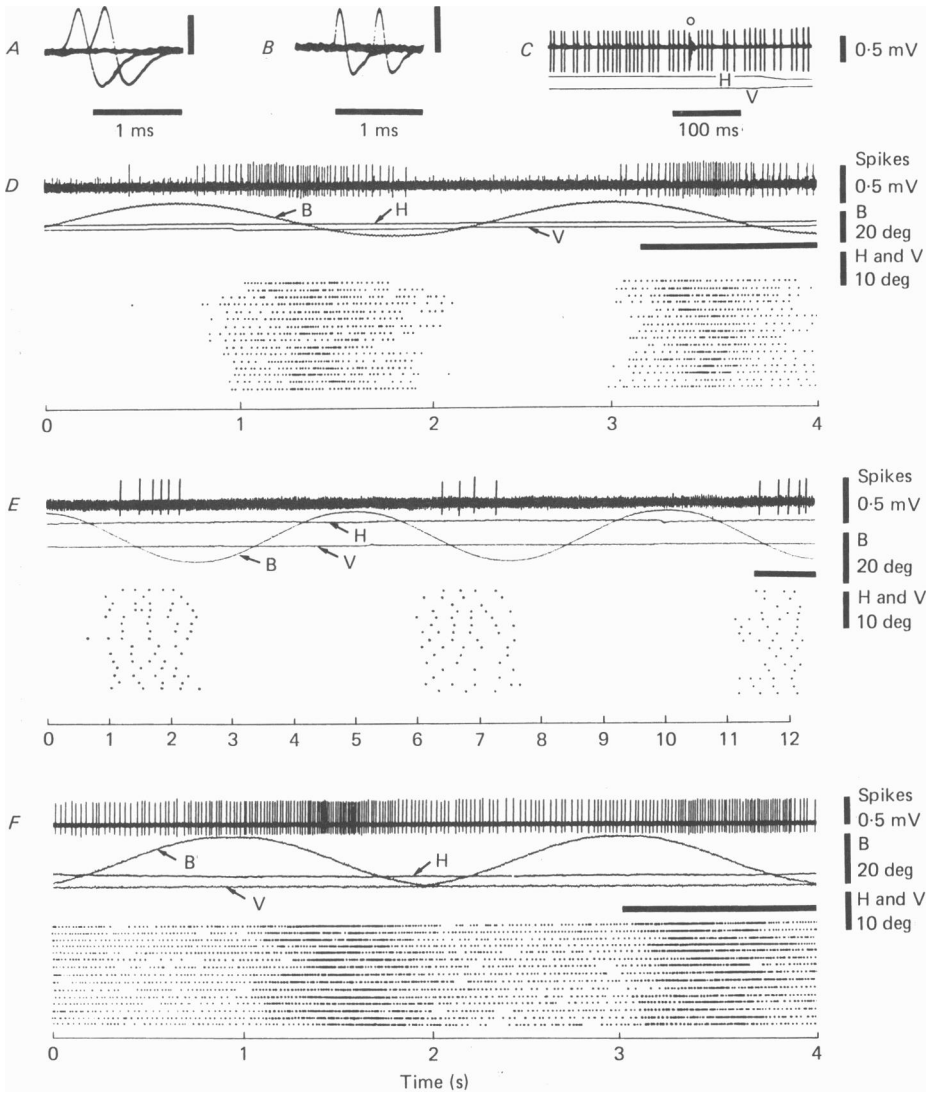

Fig. 2. *A*, an expanded film record of Purkinje-cell simple-spike discharges. *B*, discharges of a mossy fibre. Several oscilloscope sweeps were superimposed to show two spikes in both *A* and *B*. Amplitude calibration for *A* and *B*: 0·5 mV. *C*, simple- and complex-(marked with a circle) spike discharges of a Purkinje cell. *D*, discharges of a mossy-fibre unit during sinusoidal movements of the random dot background at 0·5 Hz. *E*, discharges of a climbing-fibre unit during sinusoidal movements of the same background at 0·2 Hz. *F*, discharges of an axon of a Purkinje cell during background movements at 0·5 Hz. Time calibrations for film records for *D*, *E* and *F*: 1 s. H, horizontal eye position. V, vertical eye position. B, background movements.

visual pattern and during sinusoidal smooth-pursuit eye movements are well known from the discharges of identified Purkinje cells. Because of the relatively high spontaneous firing frequency, the cyclic modulations of Purkinje-cell activity seldom returned to the zero level and a complete cycle of the sinusoidal response was superimposed on the top of the tonically increased background activity (*F*). On the other hand, the responses of mossy-fibre units were cut off during part of the inhibitory half of the stimulus cycle (*S*). The cut-off patterns, instead of the sinusoidal patterns of the Purkinje cells, characterized the responses of mossy fibres.

Two distinct groups of visual mossy fibres were found in the flocculus. Although both groups responded vigorously to sinusoidal movement of a visual stimulus during steady fixation, they seemed to signal different aspects of information about the stimulus movement. The first group (thirty-nine/sixty-four units or 61 %) responded to retinal-slip velocity, while the responses of the second group (twenty-five/sixty-four units or 39 %) reflected target velocity and showed almost identical responses regardless of whether the eyes were stationary or not. The responses of the first group were similar to those of visual neurones in the brain stem. They are designated conventionally as 'visual units'. However, the responses of the second group were different from those of any neurones known in the visual system. They signalled more highly integrated information in which each of the retinal-slip, eye, and head velocities was taken into account depending on different situations. These units are designated as 'visuomotor units'.

Visual units

This group of mossy fibres responded exclusively to retinal slip. For these units, stationary fixation was important to record clear cyclic responses to sinusoidal stimulus movements. Their responses disappeared in situations where retinal-slip velocity was nullified, for example, during optokinetic stimulation without a stationary target or during perfect tracking in darkness. These units were directionally selective. Each unit had a preference for the direction of stimulus movement and showed a vigorous response only when retinal slip was in that direction. Of the thirty-nine visual units, the preferred direction of seventeen units was to the side of the flocculus being tested (ipsilateral) and that of the remaining twenty-two units was contralateral.

The second important feature of the visual units was their sensitivity to stimulus velocity. Each unit had a range of velocity sensitivity which in some units covered that of smooth-pursuit eye movements (0–50 deg/s). However, some units were so sensitive that their discharge rates reached their peak at a stimulus velocity of less than 1 deg/s. As stimulus frequencies from 0·1 to 1 Hz were used in the present study, their responses had always saturated at our lowest frequency (peak velocity, 3 deg/s). Based on whether or not activity was related to retinal-slip velocity in the velocity range 1–50 deg/s (arbitrarily chosen), visual units could be separated into two groups. The typical responses of the two groups are shown in Fig. 3.

The difference was that the response of unit *A* (representative of twenty-six units or 67 % of the visual units) showed sinusoidal changes in firing rate, reflecting the velocity of the stimulus movement, while that of unit *B* (representative of thirteen units or 33 %) showed saturation. When the frequency of sinusoidal movements was

increased, the peak activity of the group *A* units almost invariably increased. On the other hand, the response of unit *B* quickly reached its peak and did not show a progressive elevation at the midway where the sinusoidal movements reached their peak velocity. When tested with a constant-velocity triangle-wave form movement, the response was almost identical with that of a sinusoidal movement. These units also showed analogous responses even when the frequency of sinusoidal movement was changed. The group *B* units were, therefore, sensitive to retinal slip but not to the velocity of retinal slip in the velocity range tested. Thus, the group *A* units signalled information about direction and velocity within the range of smooth-pursuit eye movements, while the group *B* units signalled information only about direction of retinal slip.

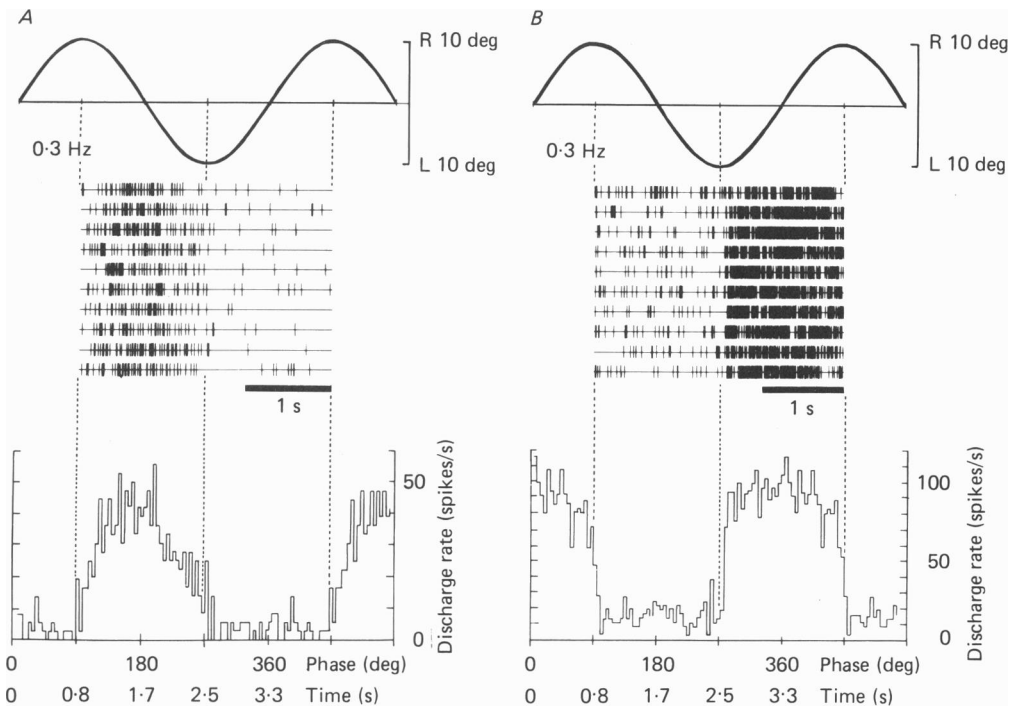

Fig. 3. Responses of visual units to sinusoidal movements of the random dot background at 0·3 Hz (peak velocity 19 deg/s) while the animal fixated on a stationary visual target. *A*, responses of a group *A* mossy fibre, showing a peak activity in the midway of the stimulus movement to the left. *B*, responses of a group *B* mossy fibre, showing saturation of discharges and a flat response. L, left; R, right.

Visuomotor units

This group of mossy fibres also responded to retinal slip. They showed cyclic modulations in response to sinusoidal movements of a visual stimulus, when both the head and eyes were stationary. The responses, however, did not necessarily represent retinal-slip velocity. They persisted even in situations where retinal-slip velocity was nullified by tracking eye movements as long as the visual stimulation was continued. In order to understand these intriguing findings, it appears useful to start with a

qualitative description of the raw data. Fig. 4 shows responses of a visuomotor unit to sinusoidal stimulus movements during stationary fixation. The film record (*A*) shows typical spikes recorded from a visuomotor mossy fibre. The raster (*B*) indicates the stimulus-to-stimulus variability and demonstrated that the responses were fairly consistent. The phase histogram (*C*) shows the firing probability in terms of the discharge rate with respect to the phase of the sinusoidal stimulus movement.

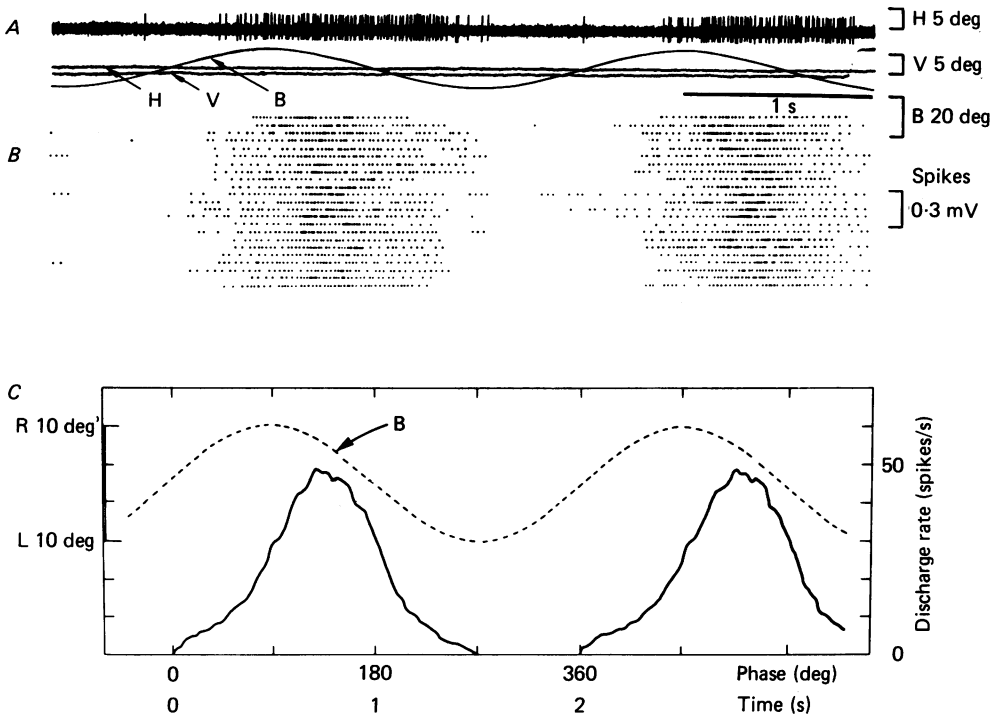

Fig. 4. *A*, original film record of a visuomotor unit. H, horizontal eye position record; V, vertical eye position record; B, movement of a random dot pattern; L, left; R, right. *B*, raster. *C*, phase histogram.

The first prominent feature of visuomotor units was a phase shift between the excitation period and stimulus movement in the preferred direction. As seen in the phase histogram (Fig. 4*C*), although the unit discharged predominantly with movement to the left, the responses started even before the change occurred in the direction of stimulus movement (turnabout). The peak activity also appeared before the peak stimulus velocity. Such a phase shift was in remarkable contrast with the visual units which responded only in the preferred direction; their responses lagged behind the occurrence of turnabouts and their peak activity also lagged behind the occurrence of peak velocity.

In addition to the phase shift, the period of excitation was usually longer than half of the stimulus cycle in most visuomotor units. These findings indicated that the response could not be produced simply by an excitatory input of retinal-slip velocity. Nevertheless, a preferred direction was evident in all the visuomotor units. Of the

twenty-five units, seventeen (68%) were excited during contralateral stimulus movement, while the preferred direction of the remaining eight units was ipsilateral.

Depending on stimulus frequency, the degree of phase shift showed a consistent change in individual units. Fig. 5 shows such a change in computer-edited data. The spike train was transformed into a continuous function representing smoothed instantaneous frequency (see Methods).

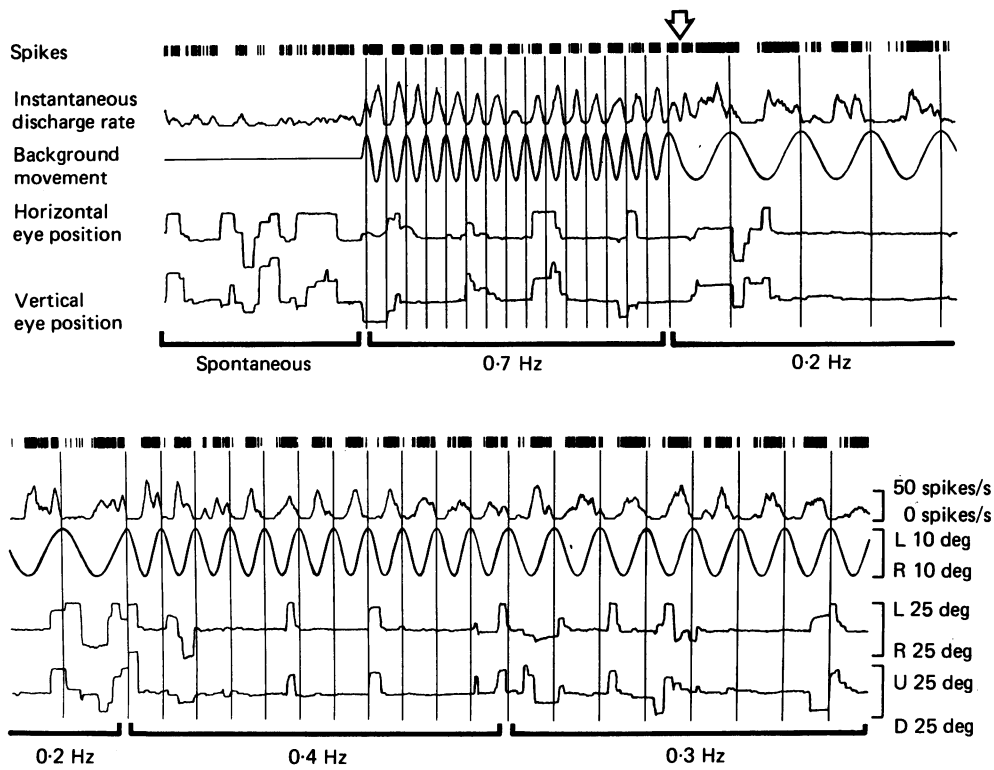

Fig. 5. Computer-edited physiological data, showing discharges of a visuomotor unit (spikes) during spontaneous eye movements in the dark (spontaneous) and during stimulation with the visual pattern moving sinusoidally in the horizontal plane at different frequencies. In order to cover the periods of various frequencies, the periods of steady fixation (which usually extends 10–20 s) were edited and shortened. By this editing, the data include more periods of eye movements than actual experimental data. Note that the discharges of the unit did not suffer modifications by eye movements. The method used to obtain the instantaneous discharge rate is described in Methods. L, left; R, right; U, up; D, down.

The following points are evident from the records of Fig. 5: (1) the cyclic modulations in activity appeared only in the presence of a visual stimulus and they reflected the regular rhythm of the movement; (2) the phase relationship between the stimulus and activity was fairly consistent within the same frequency; (3) the phase shifted consistently so that the lower the stimulus frequency, the larger the phase-lag of the activity behind the stimulus; (4) the rhythmic changes in activity were not influenced by the presence of eye movements; (5) at low stimulus

frequencies, the neural excitation was not maintained and as a result two or three peaks appeared during each cycle of 0·2 Hz stimulation; and finally (6) the effect of the preceding stimulus frequency sometimes persisted even after the frequency was altered, as indicated by an open arrow.

The phasing of the excitatory response relative to the stimulus movement in the preferred direction was determined by Fourier analyses at frequencies of 0·2, 0·3, 0·4, 0·5 and 0·7 Hz. The results are summarized in Table 1. As the stimulus velocity is mathematically 90 deg out of phase with the stimulus movement, the 85·7 deg phase lag (for 0·2 Hz stimulation) behind the stimulus corresponds to a 4·3 deg phase lead from the stimulus velocity. It is clear from Table 1 that the phase lead from the velocity increased when the stimulus frequency was increased, although the absolute time relationship between neural discharge and the velocity function remained the same (except at 0·2 Hz).

TABLE 1. The phase relationships and lead times between the responses of a mossy fibre and stimulus-velocity curves observed during sinusoidal movement of a background pattern at different frequencies

		Phase lead relative to velocity		Lead time	
Frequency (Hz)	Sample	Mean (deg)	S.D. (deg)	Mean (ms)	S.D. (ms)
0·2	(n = 9)	4·3	8·4	59·7	117
0·3	(n = 14)	13·4	15·4	124	143
0·4	(n = 13)	18·4	22·3	128	155
0·5	(n = 25)	23·4	11·7	130	65
0·7	(n = 21)	32·4	12·4	129	49

The second and perhaps more intriguing response feature of the visuomotor units was that they showed cyclic modulations even in the presence of eye movements so that the image motion of the sinusoidal background movement was not sinusoidal on the retina. Responses of a visuomotor unit during optokinetic eye movements and during steady fixation are shown in Fig. 6. The records in *A* are computer-edited data. The phase histogram in *B* shows the responses for the period without a stationary fixation target (fixation target OFF). The eyes moved frequently with the sinusoidal movement of the whole-field visual pattern. During the brief periods of the optokinetic responses, retinal-slip velocity was then minimized. The phase histogram in *C* covers the period of steady fixation (fixation target ON). Except for a brief period when the monkey looked up (open arrow), the eyes were fairly stable and retinal slip almost corresponded to the background movement. The phase histograms for periods of these markedly different situations were surprisingly similar.

The above observation suggested a possibility that the visuomotor unit signalled information about retinal-slip velocity during stationary fixation and information about eye velocity during optokinetic responses. Furthermore, it is also suggested that the visual and oculomotor inputs converge onto a unit which in turn acts like a switch and allows retinal-slip velocity to pass in one situation and allows eye

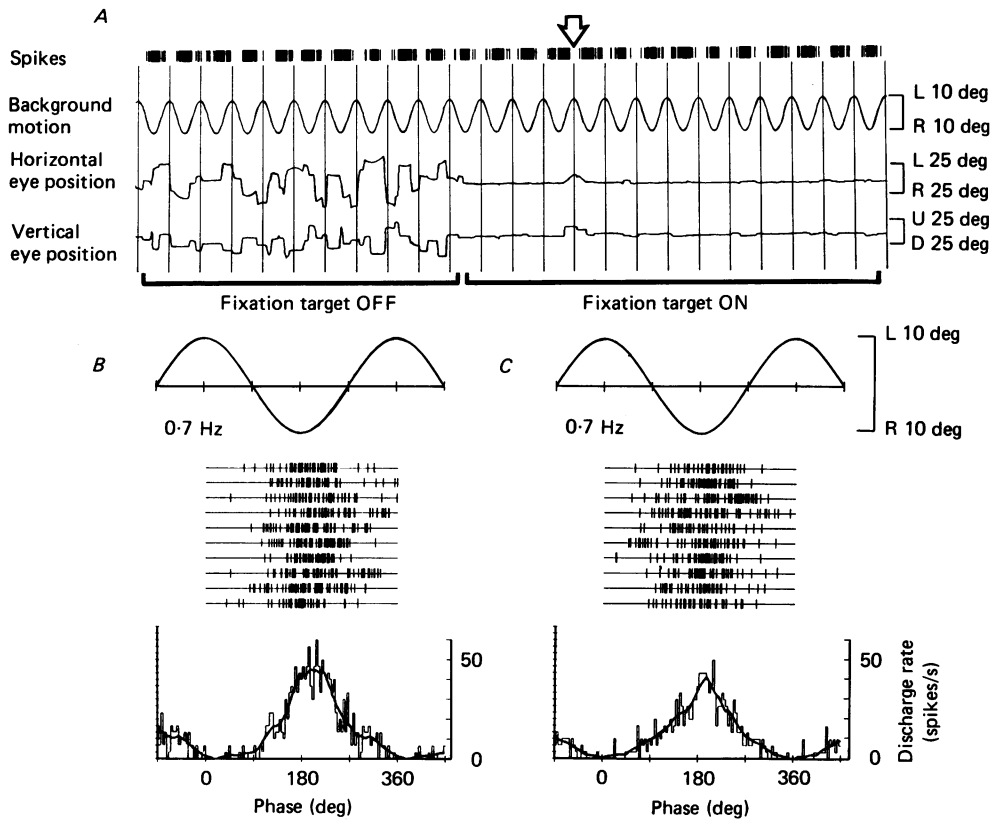

Fig. 6. *A*, computer-edited physiological data, showing discharges of a visuomotor unit (spikes) during optokinetic eye movements (fixation target OFF) and during steady fixation (fixation target ON), *B*, phase histogram for the same unit during optokinetic eye movements. *C*, phase histogram during steady fixation. L, left; R, right; U, up; D, down.

velocity to pass in the other. The following observations also support such a possibility.

In the experiments shown in Fig. 7, unit activity was studied in two situations: (1) when an animal fixated on a stationary red spot while the random dot background was moved sinusoidally (*A*) and (2) when the animal tracked the spot moving sinusoidally in darkness (*B*).

The phase histograms in Fig. 7 illustrate the modulations in the activity of a visuomotor unit. In the first situation (Fig. 7*A*), the unit discharged with stimulus movement to the right, while the optokinetic ocular responses were almost completely suppressed (gain less than 0·1). As eye velocity was minimal during this period, the modulation in activity was primarily a reflexion of retinal-slip velocity. An almost identical modulation in the neural activity was observed in the second situation (Fig. 7*B*) when there was minimal retinal-slip velocity because the eyes moved almost synchronously with the target. In this situation, the modulation in activity reflected primarily the eye velocity.

The visual receptive fields of visuomotor units were large (at least 10 deg) and

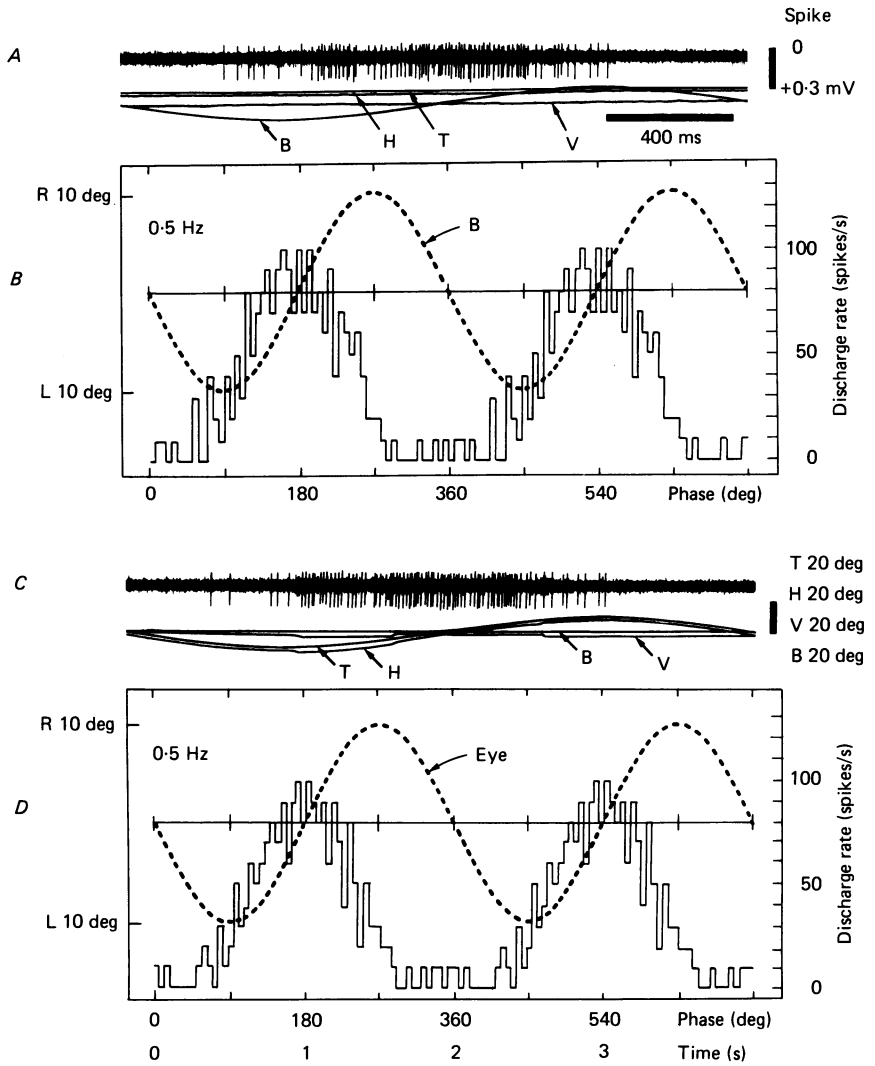

Fig. 7. Responses of a visuomotor unit to the sinusoidal movements of the random dot pattern (*A* and *B*) and discharges of the same unit during smooth-pursuit eye movements in the dark (*C* and *D*). For the phase histograms, periods of 20 cycles showing stationary fixation (or perfect smooth-pursuit eye movements) were selected from 30 cycles of the stimulus (or target) movements at 0·5 Hz and their eye positions were averaged. T, target movement; H, horizontal eye movement; V, vertical eye movement; B, background movement (the pattern was turned off in *C*); L, left; R, right.

resided outside the central 10 deg in all twenty-five units. They were in the ipsilateral hemi-field (contralateral hemi-retina) in ten units, in the contralateral hemi-field in five units, and in the bilateral periphery in the remaining ten units. In the last group, the stimulation applied to the ipsilateral visual hemi-field always elicited a larger response than that of the contralateral visual hemi-field. Although stimulation of the central retina by itself did not produce any significant responses, it seemed to have

some effect on the responses elicited by peripheral stimulation. The degree of the effects was variable but it was clear-cut in about half of the visuomotor units. An example of such a centre–periphery interaction together with responses to vestibular stimulation in the same unit is shown in Fig. 8.

The spontaneous discharge rate of this unit, evaluated when the monkey fixated on a stationary target in darkness, was 9·5 spikes/s. With sinusoidal background movement during stationary fixation, the unit responded with a peak activity of 31 spikes/s (*A*). When the same stimulus was applied through a circular window of the central 10 deg of the visual field, the response disappeared. Discharge rate might have been lower than the spontaneous level (trough activity 3 spikes/s, *B*). This possible antagonistic effect became obvious when the stimulus was presented outside the central 10 deg (*C*). The peak activity was then 47 spikes/s. The smaller response in *A* might have been caused by an inhibitory interaction between the peripheral (*C*) and the central (*B*) inputs. The same unit responded also to head rotation (*D*, *E* and *F*). The responses were obtained during suppression of the v.o.r. Since they were tested in darkness, there was no visual input.

Nine visuomotor units were successfully tested in all paradigms with rotation of the chair (see Fig. 9*B*, *E* and *F*) and vestibular inputs were demonstrated in each unit. These units signalled information about retinal slip, eye, and head velocities. This triple response was in striking contrast to that of the visual units which responded exclusively to retinal-slip velocity. In the next paradigms, discharges of a visuomotor unit were tested in response to each of these velocity signals or combinations of them (Fig. 9).

Discharges of this unit in response to smooth-pursuit eye movements in darkness (*A*) and to background movements during stationary fixation (*C*) were almost comparable with those of the unit in Fig. 7. In addition, the unit of Fig. 9 responded to vestibular inputs. It responded to sinusoidal rotation of the chair (during suppression of the v.o.r.) in darkness (*B*). The responses of the unit to retinal-slip (*C*), eye (*A*), and head velocities (*B*) were surprising similar, although the response to head velocity was 180 deg out of phase relative to the responses to retinal-slip and eye velocities. Surprisingly, the similarity in the responses was found also in the paradigms in which two of the velocity signals coexisted and must have interacted with each other. In paradigm *D*, the target was moved on the stationary background and the animal tracked it. Associated with the sinusoidal tracking eye movement, the stationary background must have slipped across the retina in the same direction. The unit discharged primarily when the eyes moved to the left. During this period, the background must have moved to the left which is the preferred direction of the unit (paradigm *C*). In paradigm *E*, the unit was tested during suppression of the v.o.r. in the presence of a stationary background. Associated with the sinusoidal head movement, there must have been retinal slip in the opposite direction. If the unit responded to both by summing its response to each presented individually, the combined response in *E* would be about twice as large a modulation as in either *B* or *C*. Although the response *E* was larger than the response to head velocity *B*, it is certainly not an algebraic sum of the responses *B* and *C*. A similar inference can be made also for the response of paradigm *F* where the chair was rotated in total darkness. The horizontal eye movement recorded during this period showed that

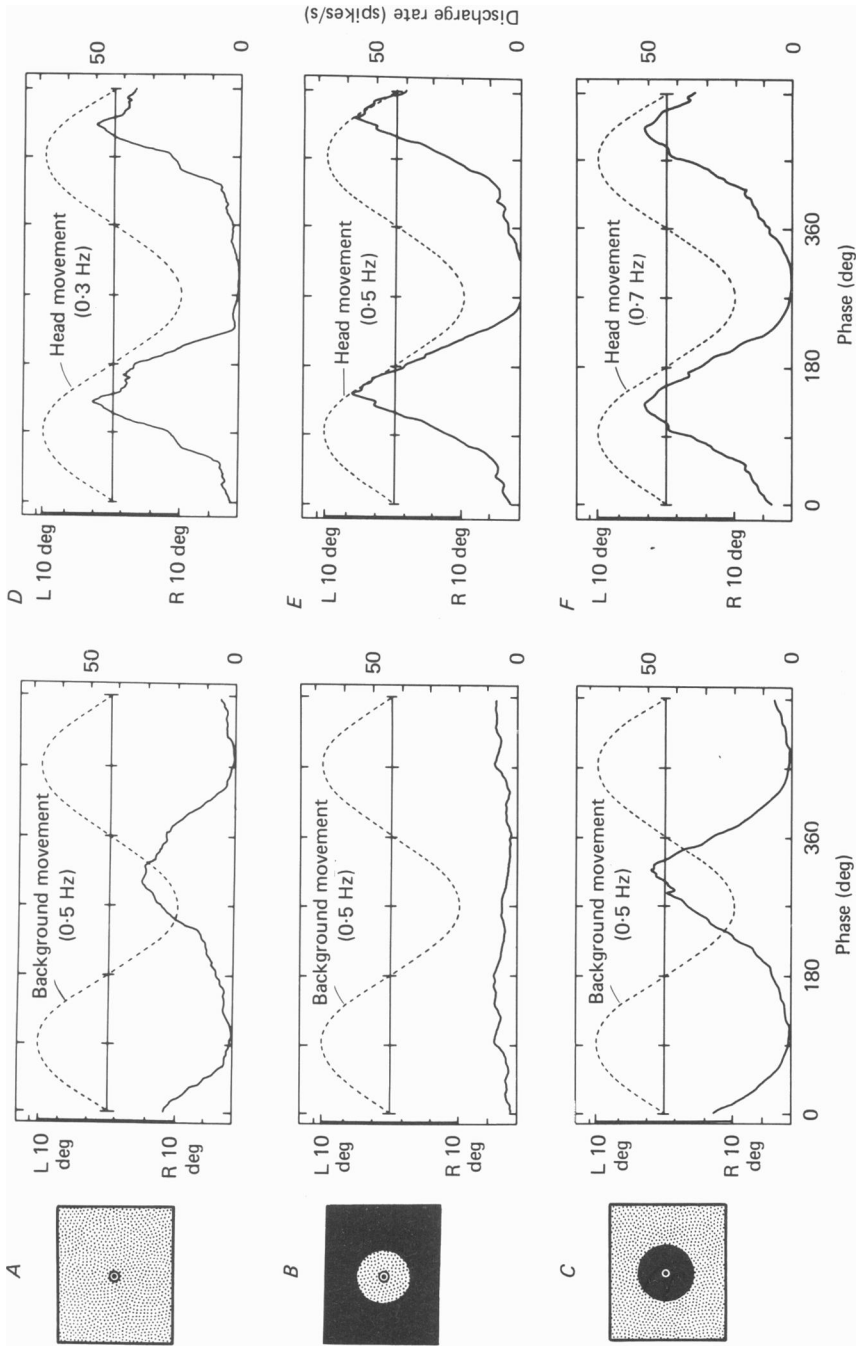

Fig. 8. Responses of a visuomotor unit during sinusoidal movements of the visual pattern at 0·5 Hz which were presented in the whole tangent screen (*A*), in the central 10 deg (*B*), in the peripheral visual field outside the central 10 deg (*C*), and modulations of the same unit during sinusoidal rotation of the chair at 0·3 Hz (*D*), 0·5 Hz (*E*) and 0·7 Hz (*F*) tested under v.o.r. suppression. L, left; R, right.

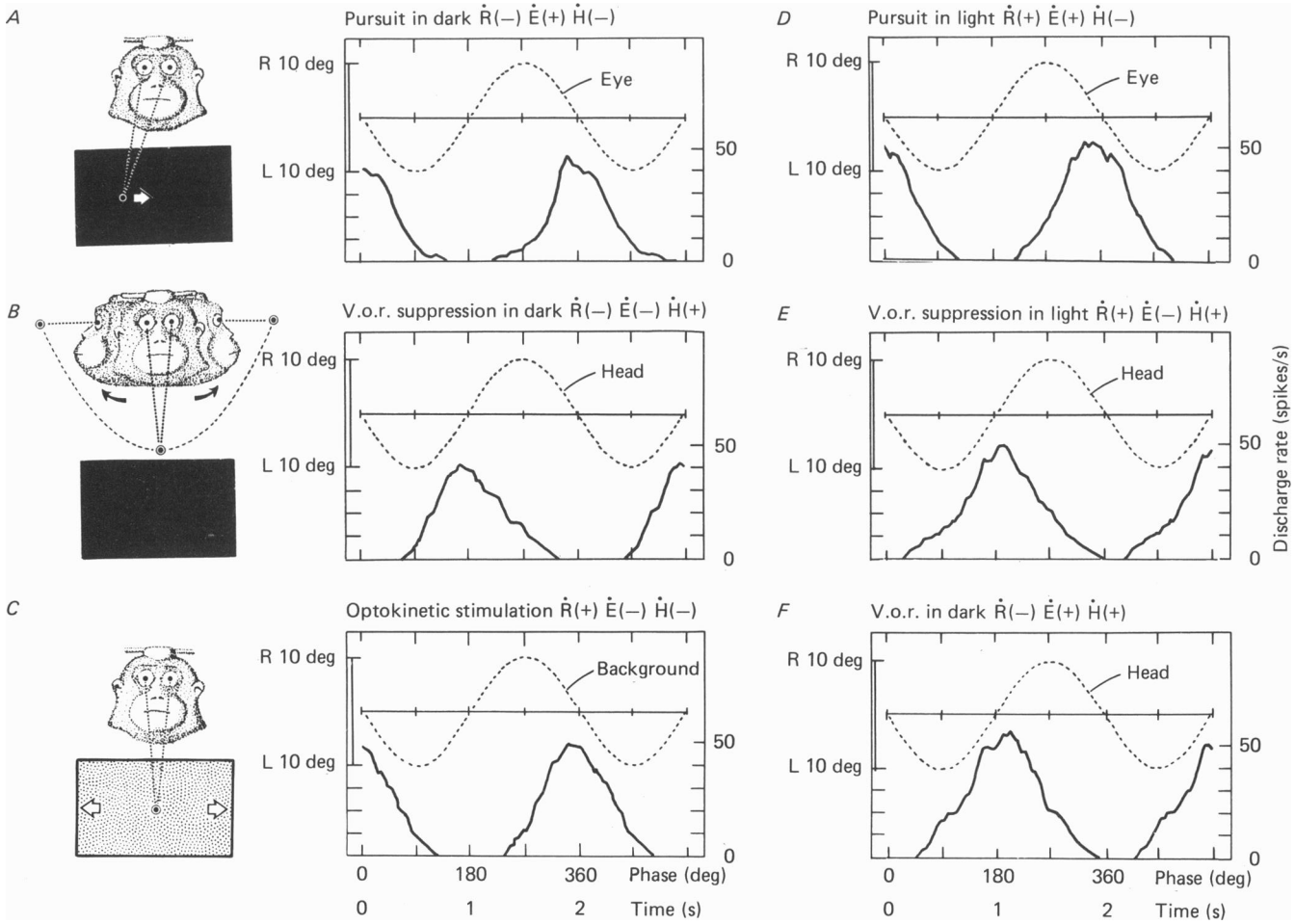

Fig. 9. Responses of a visuomotor unit during sinusoidal smooth-pursuit eye movements in the dark (*A*), during suppression of the v.o.r. in the dark (*B*), during stimulation with sinusoidal movements of the visual pattern while the animal fixated a stationary target (*C*), during smooth pursuit in the presence of the stationary random dot pattern (*D*), during suppression of the v.o.r. in the presence of the visual pattern (*E*), and during v.o.r. in darkness (*F*). $\dot{R}$, retinal-slip velocity; $\dot{E}$, eye velocity; $\dot{H}$, head velocity. Eye, Head, and Background indicate movements of the eyes, head, and background, respectively. L, left; R, right.

approximately 85 % of the time was occupied by parts of sine waves in the direction opposite to head movements. There must have been head- and eye-velocity signals whose directions are opposite to each other.

When both the head and eyes were stationary, therefore, the unit responded to retinal-slip velocity (*C*). During smooth-pursuit eye movements, the unit responded to eye velocity, regardless of whether it was accompanied by retinal-slip velocity (*D*) or not (*A*). When the head started to rotate, the unit responded primarily to head velocity, regardless of whether it was accompanied by eye velocity (*F*) or by retinal-slip velocity (*E*) or by neither of them (*B*). These behaviours of the visuomotor unit were commonly observed also in the remaining eight units tested under identical conditions.

DISCUSSION

Identification of mossy-fibre units

Perhaps the most important finding in the present study is that information concerning all of the retinal-slip, eye, and head velocities is signalled to the flocculus by single mossy fibres. Some Purkinje cells in the flocculus of the monkey receive converging inputs from the visual, oculomotor, and vestibular systems (H. Noda & T. Warabi, unpublished observation). Such a convergence, however, has not yet been described for neurones in the brain stem. It is difficult, therefore, to correlate the discharges of visuomotor units which those of any particular brain-stem neurones which might be the parent cells of the axons. Besides the fundamental question as to whether there was absolutely no contamination by multiple-unit activity, the identification of mossy-fibre units appears to be of critical importance in substantiating the present observations. Among the axonal-spike units recorded from the white matter of the flocculus, mossy-fibre units were identified by eliminating climbing-fibre and Purkinje-cell units (see Results for the details of the criteria for mossy-fibre units).

The second and even more difficult problem was to distinguish mossy fibres from granular cells. The discharge patterns of these neurones were almost identical, except for the differences in the spike configurations (axonal or cellular, see Fig. 2 *A* and *B*). Furthermore, because of their small sizes, it was not always easy to record spikes large enough to be used for the distinction. When we studied Purkinje cells (Noda & Suzuki, 1979*a*, *b*), we were not able to distinguish mossy fibres from granular cells. The micro-electrodes which were most suitable to record simple and complex spikes simultaneously from a Purkinje cell were simply too large. The discovery and manufacture of a type of micro-electrode (see Methods) which more effectively recorded mossy-fibre and granular-cell activity also facilitated the distinction of these neurones. It would have been more interesting if such a convergence had taken place first at the level of granular cells, namely within the flocculus. Histological examination, however, revealed that the recording sites of the majority of the visuomotor units were in the white matter, with a few exceptions of mossy-fibre units recorded from the granular layer.

Visual units

Visual units signalled information about retina-slip velocity to the flocculus. The velocity range covered was variable from unit to unit and it was relatively narrow as compared with those of floccular Purkinje cells (H. Noda & T. Warabi, unpublished observation). Their over-all response characteristics were similar to those commonly observed among visual neurones in the brain stem. Their responses were dependent exclusively on the movement of the visual stimulus with a relatively long delay (mean 96·5 ms, range 78–107 ms) (Noda, 1981). These units may correspond to the visual units reported by Miles *et al.* (1980) and to the three among eighty-three units described by Waespe *et al.* (1981) in alert monkeys. In both reports, however, no attempt was made to subdivide the units as mossy fibres or as granular cells.

The origin of these mossy fibres is unknown. Anatomical and electrophysiological studies have shown that the flocculus receives visual information via mossy fibres from the nucleus reticularis tegmenti pontis in rabbits (Maekawa & Takeda, 1977; Maekawa, Kimura & Takeda, 1981) and in cats (Kawasaki, Sato & Kato, 1980). Simpson, Soodak & Hess (1979) have recorded visually responsive units from the medial terminal nucleus (m.t.n.) of rabbits. The neurones had large receptive fields and preferred slowly moving textured patterns. Although the primary projection of the accessory optic nuclei to the flocculus is via climbing fibres arising from the dorsal cap of the inferior olive (Maekawa & Simpson, 1972, 1973; Alley, Baker & Simpson, 1975), it is possible, but not yet confirmed, that the m.t.n. project directly to the flocculus as a mossy-fibre input (see Brauth & Karten, 1977; Winfield, Hendrickson & Kimm, 1978). The visual mossy fibre may also originate from the dorsolateral pontine nucleus (d.l.p.n.). Suzuki & Keller (1983) have recorded units from the d.l.p.n. during periods similar to those of our retinal-slip paradigm. Their modulations were related either to the direction alone or to the direction and velocity as previously described for visual mossy fibres in the flocculus (Noda, 1981). Projections from the d.l.p.n. to the flocculus are known (Brodal, 1979, 1982; Langer, Fuchs, Scudder & Chubb, 1985).

Visuomotor units

These neurones receive converging inputs from structures related to the visual, oculomotor, and vestibular functions. Testing under situations where only one of the functions predominates, it was possible to extract the retinal-slip velocity, the eye velocity, and the head velocity, independently. Using these velocity signals, a visuomotor unit computes information about absolute target velocity and supplies it to the flocculus. When the head is stationary, for example, it provides the flocculus with information about the velocity of a visual target (or a background pattern). It is very likely that the target velocity is produced first at the level of the visuomotor units, because such a velocity signal has not been discovered in the brain-stem visual pathways. Since the responses represent the absolute target velocity, the discharges of the visuomotor units were almost identical between the two extreme situations: namely, between the stationary fixation at which the eye velocity should be zero and the smooth pursuit in which retinal-slip velocity was theoretically negligible. When the eyes lagged behind the target during smooth pursuit (or during lower-gain

optokinetic nystagmus), the target velocity corresponded to the algebraic sum of retinal-slip velocity and eye velocity because, by definition, retinal-slip velocity results from the difference between target velocity and eye velocity.

Such target-velocity information may be useful for the flocculus to produce control signals necessary to improve smooth-pursuit or optokinetic responses. The flocculus receives information about eye velocity through separate mossy-fibre channels. Such information may be considered as corollary discharges (Lisberger & Fuchs, 1978*a*; Miles *et al.* 1980). The flocculus receives also retinal-slip velocity information via visual mossy fibres (Miles *et al.* 1980; Noda, 1981; Waespe *et al.* 1981). The retinal-slip velocity, however, lags behind the stimulus velocity by approximately 100 ms (Noda, 1981). The sinusoidal eye movements observable in over-trained monkeys are almost synchronous with the target movements, indicating that the movements are largely predicted and are controlled almost instantaneously. In such a situation, the arrival of the retinal-slip velocity is too late and hence may not be useful. On the other hand, the information signalled by the visuomotor units always showed a certain phase lead relative to the target velocity. Within a limited frequency range of sinusoidal stimulation (0·3–0·7 Hz), discharges of the visuomotor units led the stimulus function by approximately 125 ms (Table 1). Such signals may be generated by the visuomotor units, based both on retinal-slip velocity of the immediate past and on corollary discharge, and might be used to predict future eye movement and to control it. This hypothesis is supported by the observation that when the frequency of the stimulus was changed, for example from 0·7 to 0·2 Hz, the signal occasionally appeared to continue for about half the cycle of the new frequency (Fig. 5, open arrow).

Although the head-velocity paradigms (Fig. 9*B*, *E* and *F*) were successfully completed in only nine out of the twenty-five units, it was our impression that all the other visuomotor units responded to the preliminary (off-the-record) tests with chair rotation in the darkness (v.o.r. paradigm, *F*). We believe therefore that head velocity may have been an important feature of the visuomotor units. In all the paradigms shown in Fig. 9, the head-velocity signal dominated the responses of the visuomotor units during rotations of the chair. When the eyes moved with the head over the stationary background (v.o.r. suppression in the light, *E*), there was retinal slip in the direction opposite to the chair rotation. Theoretically, the response *E* must be the algebraic sum of the head velocity (*B*) and the retinal-slip velocity (*C*) but with *B* and *C* 180 deg out of phase with one another and, therefore, the response *E* should be larger. Analogous inference may be drawn for the v.o.r. paradigm (*F*) where the head movement is associated with eye movements in the opposite direction. Here again, the response *F* was not an algebraic sum of responses *B* and *A* in the opposite direction.

A more complex situation is seen in paradigm *D* where there were two conflicting signals. One was related to the target velocity (or to eye velocity as it tracked) and the other was related to the velocity of background motion in the opposite direction that was induced during tracking. If these signals were treated by the unit simply as two stimuli, there should be two responses: one at about 180 deg and the other at about 360 deg in the phase histogram *D*. In this situation, the unit responded only to the target–eye velocity.

Discharges of visuomotor units during paradigms involving paired stimuli in

combination clearly cannot be explained by algebraic summing of the discharge patterns seen in response to the individual stimuli applied in isolation. The visuomotor units may serve therefore as a switching mechanism, rather than a summing mechanism, between these different inputs. This switching may in turn be controlled by other factors such as attention so that the same visual stimulus may have different effects on the responses depending on whether or not the stimulus is used for the control of a motor performance. At present, however, we do not have any electrophysiological evidence to explain how the target-velocity signal is computed at the level of synapses or at any other part of a visuomotor neurone. It is also not known by which mechanism such a psychological factor might influence impulse generation in the parent cell of the mossy fibre.

Neurones showing the behaviour of the visuomotor units have not yet been described in the brain stem. However, this is simply because brain-stem neurones have not yet been tested in the paradigms designed to extract individual velocity signals. We discovered such absolute target-velocity units also among both mossy fibres and Purkinje cells in lobulus VII of the cerebellar vermis (Kase, Noda, Suzuki & Miller, 1979). Although these units were not tested with the vestibular paradigms, the response characteristics of the mossy fibres such as the sensitivity to retinal-slip velocity, the phase relation to stimulus velocity, or the switching behaviour (representing either eye velocity or retinal-slip velocity depending on the situation) were almost identical with those of the visuomotor units in the flocculus. Under appropriate testing conditions, therefore, the parent cells of these mossy fibres must be discovered in structures which project mossy fibres to the posterior vermis and to the flocculus.

Many neurones recorded from the vestibular nuclei of the alert monkeys responded to both visual and vestibular stimulation (Henn, Young & Finley, 1974; Waespe & Henn, 1977, 1978). In fact, many of these neurones may project to the flocculus. Waespe *et al.* (1981) have recorded eighty-three input units from the flocculus of the monkey (three of these may correspond to our visual units). The responses of these input units (including both granular cells and mossy fibres) during vestibular, optokinetic, and combined visual–vestibular stimulations were very similar in many respects to the vestibular neurones previously described by Waespe & Henn (1977, 1978). In addition, Miles (1974) has recorded neurones from the medial and superior vestibular nuclei that modulated their firings sinusoidally in an experimental paradigm which was identical to our smooth-pursuit paradigm (Fig. 9*A*). The same neurones responded also to vestibular inputs tested during suppression of the v.o.r. It is not clear, however, whether his experiment was performed in the light or in the dark. Nevertheless, the responses recorded by Miles (1974) showed the cut-off pattern which characterized our visuomotor mossy-fibre units. Because our units shared common features with the vestibular responses studied during the visual and vestibular interaction by Waespe & Henn (1977, 1978) and during the v.o.r. by Miles (1974), it is possible that the same group of vestibular neurones may have been involved. It is most likely, therefore, that the parent cells of our visuomotor mossy fibres are vestibular neurones.

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