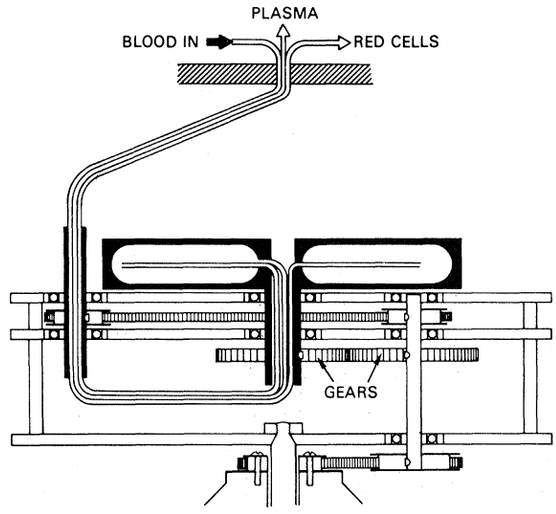

Fig. 2. Design of the centrifuge: the frame of the centrifuge body consisting of three horizontal plates carries three rotary structures—central bowl, countershaft (right), and tube-supporting hollow shaft (left). The coupling of the lower pulley of the countershaft to the stationary pulley on the motor housing causes counterrotation of the countershaft with respect to the rotating frame. This motion is then conveyed to the central bowl by 1 : 1 gearing to double the angular velocity of the bowl. The motion of the countershaft is also transferred to the tube-supporting hollow shaft by means of the pulley coupling (1 : 1 ratio). Thus the system satisfies all requirements indicated in Fig. 1.

boscopic illumination. Three silicone rubber tubes from the rubber bag are led down through the center of the centrifuge bowl, then upward through the hollow shaft, and finally through the center hole of the centrifuge cover where they are tightly supported. When properly balanced, the centrifuge bowl can be operated at speeds up to 2000 rev/min.

In order to demonstrate the capability of the apparatus, heparinized (1.5 mg/kg) sheep blood was introduced into the centrifuge directly from the animal (weight, 34 kg) while effluents of the plasma and red blood cells were returned (after sampling) to the animal. The flow rates through the individual lines were controlled by two roller pumps, one set on the whole blood line and the other on the plasma return line, the third line having a flow equal to the difference between the two pumps. With a constant feed rate of 60 ml/min, plasma free of red blood cells was harvested at 12 ml/min at 1000 rev/min or 18 ml/min at 1300 rev/min. During 12 hours of continuous flow of plasma at 18 ml/min, blood and plasma samples were collected at intervals so that changes in the platelet counts could be studied. Our results showed a 50 percent reduction in the blood platelet count within the first hour, and a reduction to 30 percent of the base line values by the 12th hour of operation without any evidence of red blood cell hemolysis. These results are similar to those reported from studies in which a membrane lung was used in a similar perfusion system without a centrifuge (*2*).

The scheme presented here provides a broad application to plasmapheresis, cell washing and elutriation, zonal centrifugation, and countercurrent chromatography (*3*).

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Visual Tracking and the Primate Flocculus

Abstract. *Purkinje cells in the primate flocculus discharge specifically in relation to visual tracking, effectively generating a velocity profile of the target during pursuit. It is suggested that these neurons supply oculomotor centers with the velocity command signals needed to support pursuit eye movements.*

Primates have the remarkable ability to fixate and track small moving targets; selected images are thereby brought onto the fovea and stabilized for detailed visual processing. The tracking function is undertaken by the smooth pursuit system, which is believed to operate, at least in part, like a velocity servo (*1*). Responding to the slip of the target's retinal image, this system furnishes the velocity command signals needed by oculomotor centers to move the eyes at a rate which will nearly match that of the target. Although head movements occur frequently during tracking, they have remarkably little net influence on the pursuit system's task due to the action of the vestibulo-ocular reflex (*2*); this system senses head rotations and signals to the oculomotor centers to forge compensatory eye movements which offset the head motion and thereby stabilize the eyes in space. The ocular pursuit system thus deals with the actual motion of the target in space independently of any accompanying motion of the head. We now show that Purkinje cells in the primate flocculus fire specifically in relation to visual tracking; by introducing controlled head rotation into the tracking situation, we have demonstrated that Purkinje cell discharges can describe the track target's actual velocity. We propose that these cells constitute part of the smooth pursuit system and provide oculomotor centers with the target velocity information required for pursuit eye movements; we also cite data from clinical and lesion studies to support this thesis. We further suggest that the flocculus constructs this velocity profile of the track target from visual, vestibular, and oculomotor inputs, which we have demonstrated in the flocculus.

Single unit recordings were made in both flocculi of the awake rhesus monkey trained on a visual fixation task according to the paradigm devised by Wurtz (*3*). Microelectrodes were positioned with an implanted cylinder and attached microdrive (*4*). The monkey was seated in a special primate chair that could be oscillated about a vertical axis by a torque motor servo system, providing adequate vestibular stimulation. The animal's head was secured to the chair by implanted bolts, and its eye movements were recorded by d-c electro-oculography. A variety of small fixation targets were projected on a translucent screen; the monkey viewed the screen through a double-mirror optical system arranged like a horizontal periscope and mounted on the chair. The first mirror in this system was connected to a modified pen galvanometer immediately in front of the monkey's right eye (the left eye was masked), and could be used to oscillate the visual field back and forth in the horizontal plane. The smooth target movements needed to elicit tracking were obtained by driving the galvanometer power amplifier with a voltage waveform generator. Electrolytic marking lesions were made by passing current through the microelectrode; these served to locate specific recording sites and facilitate reconstruction of the electrode tracks.

Most of the unit activity in the flocculus attributable to Purkinje cells because of associated complex spikes (CS's) showed modulation of the so-called simple spike (SS) firing during visual tracking (32 of 43

such units observed in this situation). Figure 1 shows the firing of one such unit recorded in the left flocculus while the monkey tracked a sinusoidally oscillating target; there is clear modulation in phase with eye and target velocity, and, as usual with most units of this type (29 of 32), firing increased during the ipsilateral phase of such movements. Although the whole background moved with the target in this example, essentially the same modulation was seen when the target moved in otherwise totally dark surroundings or even against a stationary featured background; thus, we are dealing with events linked to foveal smooth pursuit mechanisms and any peripheral optokinetic influence seems to be minor if not irrelevant. This particular unit was unusual in that its activity also increased slightly with ipsilateral fixations. While only a few units shared this weak, eye-position sensitivity, most showed some slight transient change in SS firing during saccadic eye movements; this was usually a brief pause, although in occasional units a slight burst accompanied saccadic movements in some directions. In four units there was a reciprocal relation between CS and SS firing, such that the already infrequent CS's (average rates less than 1 per second) ceased completely during periods when SS rates were elevated. Such inverse relations have been noted by others (*5*). Complex spikes were not observed to fire consistently in relation to any other parameter we scrutinized (*6*).

In order to see if these firing patterns were tied to some aspect of the stimulus (target movement) or the observed response (eye movement), or both, we introduced an additional stimulus (movement of the monkey's head) that we believed would dissociate these inputs and outputs without disrupting the tracking process (*2*). Figure 2 shows for the unit described in Fig. 1 that the track-related firing is linked to target movement and can be readily dissociated from eye movements; furthermore, the movement of the target relative to the earth-fixed surroundings rather than to the head is the primary determinant of firing. Thus, throughout the paradigms of Fig. 2, unit firing correlates with the target's velocity regardless of any imposed motion of the head. In the situation described in Fig. 2A, the target was mounted on the chair, and the animal and target were oscillated together; in this special situation, the pursuit and vestibular subsystems essentially cancel one another, and the eyes remain stationary in their orbits. This drastic alteration in the pattern of eye movements during tracking has little effect on the unit's pattern of firing, which remains tied to the track target's velocity, increasing its rate during the leftward movements. The unit's depth of modulation is very similar in Figs. 1 and 2A; this probably reflects the similarity of the peak target velocities (approximately 18° per second) even though frequencies and amplitudes were different.

When the fixation target is stationary, that is, at zero velocity, merely oscillating the monkey fails to produce any consistent modulation (Fig. 2D) (*7*). In Figs. 2, B and D, target movements were coupled to chair movements by driving the galvanometer-mounted mirror—through which the monkey viewed the targets—with the feedback signal from the chair servo; this allowed us to move the chair either in phase with the

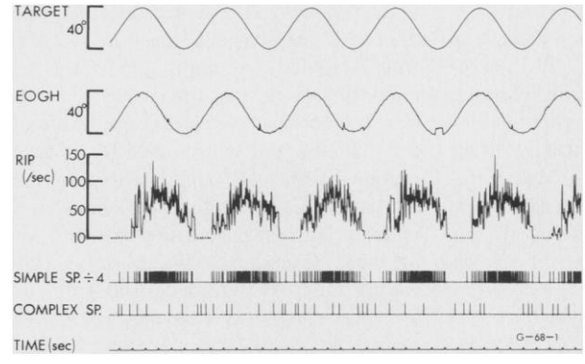

Fig. 1. Purkinje cell activity in the left flocculus during visual tracking. The target was moving in the horizontal plane, upward deflection indicating rightward movement. Simple and complex spike firing are separately displayed as pulses. Abbreviations: EOGH, horizontal electro-oculogram; RIP, reciprocal interval plot indicating instantaneous frequency for the simple spike only. (Target movements: ± 19° at 0.15 hertz; monocular viewing, right eye.)

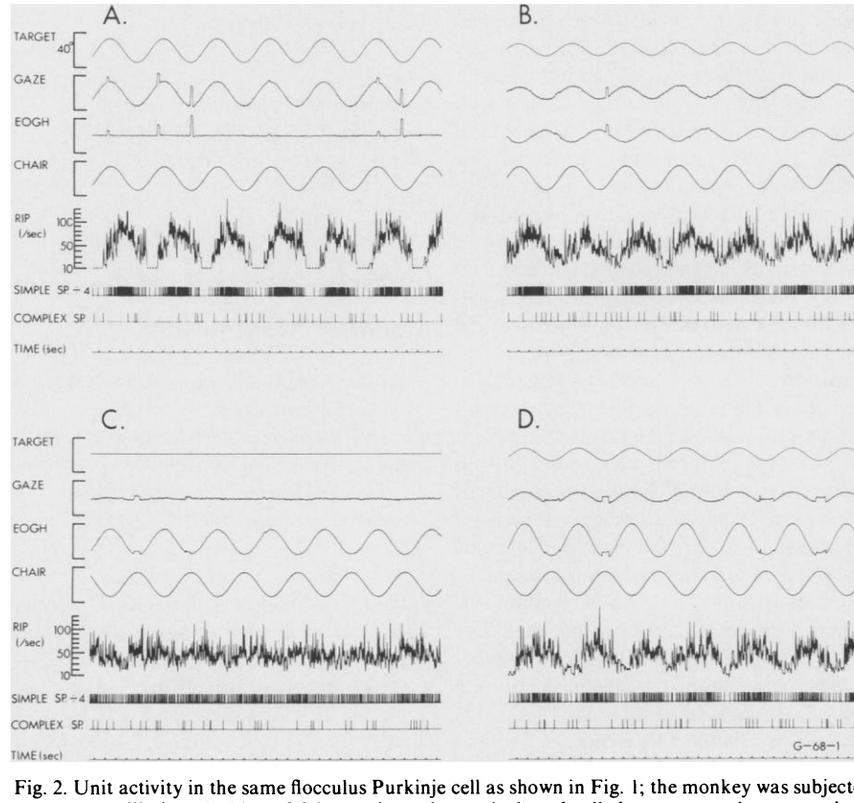

Fig. 2. Unit activity in the same flocculus Purkinje cell as shown in Fig. 1; the monkey was subjected to rotary oscillations (±14° at 0.2 hertz about the vertical) and called upon to track targets whose movements were coupled in various ways to chair movement. Gaze monitors were synthesized by summing the horizontal oculogram (EOGH) and the chair position signals; the various monitors are displayed at the same gain, upward deflections representing rightward movements; other monitors as in Fig. 1. Target, gaze, and chair positions are all relative to earth-fixed surroundings. The monkey viewed a screen and targets through a double-mirror optical system mounted on the chair, which could be used to oscillate the animal's visual field in or out of phase with the chair movement. Monocular viewing throughout (right eye). (A) The mirror was stationary and the target was mounted on, and therefore moving with, the chair; effective target movement: ± 14°, in phase with the chair. (B) A stationary target was viewed through a mirror oscillating in phase with the chair; effective target movement: ± 7°, in phase with the chair. (C) A stationary target was viewed through a stationary mirror; effective target movement: ± 0°. (D) A stationary target was viewed through a mirror oscillating 180° out of phase with the chair; effective target movement: ±7°, 180° out of phase with the chair.

target (Fig. 2B) or 180° out of phase with it (Fig. 2D). In both situations the unit continued to modulate in relation to the track-target's velocity, increasing its rate with the leftward movement. All 13 track-related Purkinje cells tested on all of these paradigms showed essentially the same pattern of firing. In the few units examined under conditions in which the movements of the target and chair were completely dissociated, track-target motion relative to earth-fixed surroundings was still the crucial factor. Irregularities in firing which are apparent as noise in the reciprocal interval plots tend to obscure the correlation with target velocity; they may reflect microvariations in the monkey's performance which are beyond the resolution of our oculogram recordings but are nonetheless significant.

That these neural correlates of visual tracking in the primate flocculus are probably not mere epiphenomena but may instead reflect some vital role in the programming of pursuit eye movements is suggested by the deficits which follow lesions in this area. Severe, and relatively specific, impairments of smooth pursuit eye movements are often associated with cerebellar atrophy in man (*8*), and total cerebellectomy in the mature monkey results in the permanent loss of such eye movements (*9*). Deficits in optokinetic responses after bilateral flocculectomy in monkeys have been reported, but, unfortunately, the animals were not examined specifically for pursuit eye movements of the kind under consideration here (*10*).

We hypothesize that Purkinje cell output from the primate flocculus provides oculomotor centers with target velocity information essential for visual tracking and represents an output of the smooth pursuit subsystem. In addition, we have some data which may help explain how the flocculus generates these velocity command signals. Most units in the flocculus showed no evidence of a CS, and we assume that they represent one or another of the various input elements known to influence Purkinje cells. The majority of such units fired vigorously in relation to saccadic eye movements often with both transient and tonic components but no apparent special concern with tracking; others seemed to be driven by vestibular inputs, modulating nicely in phase with chair (that is, head) velocity. We became aware of a class of visually driven units lacking CS's; these were especially sensitive to retinal image slip in the region of the fovea and often were more responsive to ipsilateral target movements. Firing of these units may be the putative error signal that ultimately sustains pursuit eye movements. These units closely resemble those recently described for the monkey's nucleus of the transpeduncular tract (*11*), a part of the accessory optic system; at least in the rabbit, this tract projects to the flocculus (*12*). Signal processing in the pursuit system may require a precise velocity representation of the target (*13*); we propose that this is the function of the Purkinje cells in the primate flocculus. A true neuronal facsimile of the track target's absolute velocity would require the summing of three signals: velocity of the target's retinal image (target motion relative to eye motion), eye velocity (eye motion relative to head motion), and head velocity (head motion relative to earth motion); we know that information concerning the first and last of these reaches the flocculus, and the second might easily be derived from the numerous inputs related to eye movements. A possible complication arises if the system has predictive capabilities, since the tracking waveforms contrived in our study were usually highly periodic (sinusoids and linear ramps).

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17 March 1975; revised 27 May 1975

Antimitotic Activity of the Potent Tumor Inhibitor Maytansine

Abstract. *Maytansine, at 6×10^{-8}M, irreversibly inhibits cell division in eggs of sea urchins and clams. It causes the disappearance of a mitotic apparatus or prevents one from forming if added at early stages. Maytansine does not affect formation of the mitotic organizing center but does inhibit in vitro polymerization of tubulin. Maytansine and vincristine inhibit in vitro polymerization of tubulin at about the same concentrations, but vincristine is about 100 times less effective as an inhibitor of cleavage in marine eggs.*

Maytansine, a novel ansa macrolide (Fig. 1), isolated from various *Maytenus* species, is an antitumor agent (*1*) that significantly inhibits mouse P-388 lymphocytic leukemia in dosages of micrograms per kilogram of body weight, and is active over a 50- to 100-fold dosage range. Maytansine also shows significant inhibitory activity against the L-1210 mouse leukemia, the Lewis lung carcinoma, and the B-16 melanocarcinoma murine tumor systems. This agent is being tested toxicologically in preparation for clinical trials (*2*).

We report now on the antimitotic effects of maytansine. At a concentration of $6 \times 10^{-8}M$, it totally inhibited cleavage in sea urchin eggs when applied at fertilization (*3*). At $4 \times 10^{-8}M$, 10 to 20 percent of the eggs divided (although cleavage was somewhat irregular). The remaining eggs formed irregular furrows that either did not separate equal-sized blastomeres or they subsequently retracted. When the eggs were treated with $10^{-8}M$ (or less) maytansine, the cleavage time, cleavage pattern, and later development were normal.

The critical time in egg development for inhibition of cleavage by $10^{-7}M$ maytansine was determined by adding the drug at 5-minute intervals, from the time of fertilization to first cleavage. Cleavage was totally inhibited when the drug was added at any time during the first half of the cleavage period; after that, an increasing number of cells went through some form of cleavage. However, even when the drug was added 10 minutes prior to cytokinesis, approximately 40 percent of the eggs did