JOURNAL OF NEUROPHYSIOLOGY
Vol. 53, No. 2, February 1985. *Printed in U.S.A.*

Effects of Unilateral Lesions of the Flocculus on Optokinetic and Vestibuloocular Reflexes of the Rabbit

N. H. BARMACK AND V. E. PETTOROSSI

Department of Ophthalmology, Neurological Sciences Institute, Good Samaritan Hospital and Medical Center, Portland, Oregon 97209; and Institute of Human Physiology, Catholic University of the Sacred Heart, Rome, Italy

SUMMARY

1. The horizontal optokinetic reflex (HOKR) and the horizontal vestibuloocular reflex (HVOR) were tested in 21 rabbits before and after unilateral lesions were made in the left cerebellar flocculus.

2. The immediate effect, observed within 15 min following placement of a floccular lesion, was a conjugate nystagmus with the slow phase toward the side opposite to the lesion when the animal was placed in total darkness. This spontaneous nystagmus lasted from several hours to two days depending on the extent of damage to the flocculus. It was reversed in sign if the subjacent vestibular nuclei or vestibular nerve were damaged by the operation, and it was totally absent if the unilateral floccular lesions were made in rabbits that had been bilaterally labyrinthectomized.

3. The spontaneous drift of the eyes observed immediately postoperatively caused a bias in measurement of the HVOR that was dependent on the frequency of vestibular stimulation.

4. When measured 50 days postoperatively the HVOR had a normal gain and normal bias. When measured 50 days postoperatively the monocular HOKR (posteroanterior stimulation of the left eye) was significantly reduced in gain at stimulus velocities below 5°/s.

5. A quantitative anatomical analysis of the degeneration of inferior olivary neurons caused by lesions of the flocculus demonstrated contralateral cell loss of as much as 65% of the dorsal cap neuronal population.

6. These data reveal a permanent deficit in the HOKR, but not the HVOR, following unilateral floccular lesions and are consistent with the idea that the flocculus contributes to the regulation of the low-velocity eye movements through the inhibitory modulation of the activity of the subjacent vestibular nuclei.

INTRODUCTION

The inhibitory Purkinje cell output of the cerebellar flocculus onto the subjacent vestibular nuclei (2, 16, 26) places the flocculus in a strategic position for modulating reflexes mediated by the vestibular nuclei. These reflexes include the vestibuloocular reflex (VOR), the optokinetic reflex (OKR), and cervicoocular reflex (COR). The flocculus receives visual, vestibular, and neck proprioceptive information via climbing fiber afferent pathways that originate from the contralateral inferior olive (1, 3, 6, 14, 20, 33, 39, 45, 46). Visual and vestibular inputs also converge at the level of the vestibular nuclei (22, 29) and because the vestibular nuclei project to the flocculus these inputs can also reach the flocculus indirectly as mossy fiber projections that originate from the vestibular nuclei (11) as well as from other brain stem regions (34, 35).

Visual climbing fibers originate from the dorsal cap of the inferior olive, cross the midline, and synapse on Purkinje cells in the

contralateral flocculus (1, 6, 31, 33). These climbing fibers receive directionally selective information mediated by the ipsilateral nucleus of the optic tract that originates from the contralateral eye (6, 23, 39). Electrical stimulation of the right dorsal cap evokes low-velocity conjugate rightward eye movements, implicating the olivocerebellar circuitry in a feedback loop in which retinal slip of low velocity is nulled by evoking eye movements that reduce the retinal slip (7). These findings are consistent with the obser-

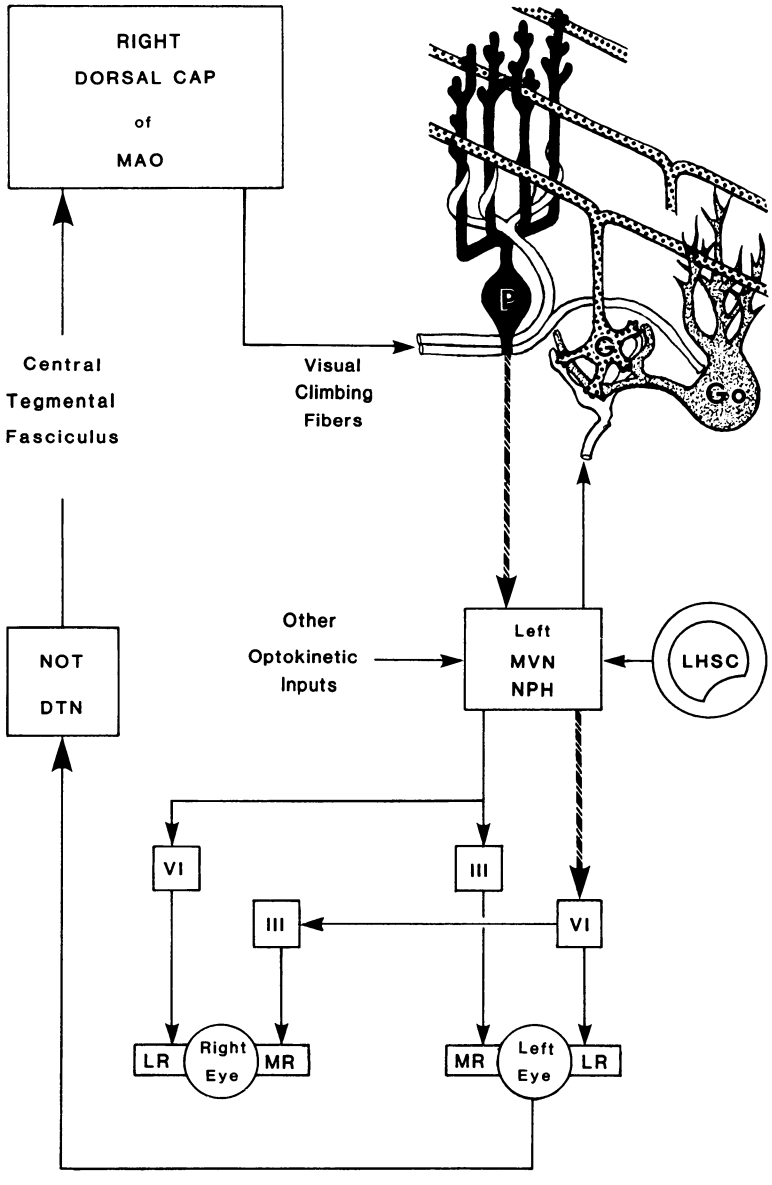

FIG. 1. Schematic representation of visual olivocerebellar pathway relevant to control of horizontal eye movements. *Dashed lines* indicate pathways that are known to be functionally inhibitory. DTN, dorsal terminal nucleus; NOT, nucleus of optic tract; MAO, medial accessory division of inferior olive; LHSC, left horizontal semicircular canal; MVN, medial vestibular nucleus; NPH, nucleus prepositus hypoglossi; III, oculomotor nucleus; VI, abducens nucleus; LR, lateral rectus muscle; MR, medial rectus muscle; P, Purkinje cell; G, granule cell; and Go, Golgi cell.

vation that lesions of the right dorsal cap temporarily cause a leftward drifting eye movement in the dark and permanently reduce the gain of the HOKR for posteroanterior optokinetic stimulation of the left eye (8).

In order for this olivocerebellar circuitry to evoke eye movements of the correct direction it is necessary to assume that small increases in climbing-fiber responses (CFRs) cause larger decreases in the frequency of simple spikes (SSs) in floccular Purkinje cells. There is ample electrophysiological evidence demonstrating this antiphasic behavior of CFRs and SSs (9, 10, 12, 32, 36). One possible anatomical substrate for this interaction might be Golgi interneurons that inhibit mossy fiber inputs at the level of the granule cell glomerulus (17) (Fig. 1). Golgi cells are the only cerebellar interneurons that receive collaterals of CFs (15, 21).

In the specific case of the visual olivocerebellar pathway, posteroanterior optokinetic stimulation of the left eye would excite CFs that originate in the right dorsal cap and evoke an increase in CFRs in the left flocculus. This increase in CFRs would cause a net reduction in Purkinje cell response (CFRs + SSs), disinhibiting the subjacent left vestibular nuclei. The consequent decreased inhibition of the left vestibular nuclei would cause a conjugate movement of the eyes to the right, thereby reducing the posteroanterior retinal slip of the left eye (Fig. 1).

A further evaluation of the role of the flocculus in the modulation of reflexes could be made by examining the effect of unilateral lesions of the flocculus on spontaneous and reflexive eye movements. According to the "wiring diagram" based on previous experiments (Fig. 1), a lesion of the left flocculus should cause disinhibition of the subjacent left vestibular nuclei and consequently a bias of the eyes to move to the right, as if the climbing-fiber pathway originating from the right dorsal cap was being electrically stimulated. Whether such a rightward bias would be evident in both the HVOR or HOKR and whether such a bias would be permanent would be of particular interest.

The present experiment was undertaken to assess the effect of lesions of the left flocculus on eye movements of the rabbit. Our principal finding is that unilateral lesions of the flocculus cause a permanent decrease in the gain of the horizontal optokinetic reflex when optokinetic stimulation is delivered monocularly to the eye that projects to the damaged flocculus. These findings have been reported in preliminary form (4, 5).

METHODS

Surgical procedures

Twenty-one rabbits, pigmented and albino, were used with equivalent results in the present experiment. In preparatory operations the rabbits were anesthetized with intramuscular injections of ketamine hydrochloride (50 mg/kg) and paraldehyde (0.5 ml/kg). Two stainless steel screws (10–32) were anchored to the calvarium with four smaller peripherally placed stainless steel screws (2–56) and dental cement. The two larger screws mated with a steel rod by which the head of the rabbit was restrained subsequently without obscuring the visual field and to which an eye-position transducer could be attached.

Eye-position recording

Eye position was measured with an infrared light projection technique. The eye was anesthetized topically with proparacaine hydrochloride. A small suction cup bearing a light-emitting diode (LED) was attached to the anesthetized eye. The LED projected a narrow beam of infrared light onto a photosensitive X-Y position detector (United Detector Technology, SC-50) that was fixed relative to the head and located 3–5 mm from the tip of the LED (G. E., SSL-315). The photosensitive surface gave a continuous X-Y indication of the position of the incident centroid of infrared light. The system was calibrated by moving the eye on which the LED was mounted through known angular displacements. This system had a sensitivity of 60 mV/degree and was linear to within 5% for deviations of the eye of ±15°. Eye velocity was measured by electronic differentiation of the eye-position signal.

Vestibular stimulation

Rabbits were mounted on a biaxial, servocontrolled rate table. The previously implanted head bolts mated with a steel rod that fixed the head at the center of rotation of the table. The body was held in a cradle firmly encased in foam rubber. The waveform amplitude and frequency of rotation could be controlled independently. The frequency response of the table was determined by measuring the table position with a servopotentiometer at different frequencies of sinusoidal (±10°) inputs. The frequency response of the table in the horizontal plane was of constant amplitude at input frequencies of <0.8 Hz.

Optokinetic stimulation

The rate table was located 55 cm from the center of a rear-projection tangent screen that subtended 70 x 70° of visual angle. The optokinetic stimulus was rear projected onto the screen by beaming the image projected by a 35-mm slide projector off a first surface mirror that was mounted on an electroencephalogram (EEG) pen motor. The direction and velocity of optokinetic stimulation was controlled by supplying an appropriate voltage ramp to the pen motor. A rear-projection tangent screen is truly tangent at only one point on the optical axis. Consequently, linear distances and angular distances are not equivalent, and a "constant-velocity" target will in fact have a lower angular velocity at the extreme edges of the screen. For the present tangent screen, the velocity of the optokinetic stimulus was ~12% lower for the most peripheral 5° relative to the most central 5° subtended by the tangent screen. Our calibrations of optokinetic stimulus velocity were based on the time required for a point to traverse the entire tangent screen and therefore represent average velocities. The rabbit was aligned with respect to the tangent screen so the optokinetic stimulus subtended the central 70° of the eye being stimulated. This alignment was important because there are differences in the efficacy of optokinetic stimulation delivered to the anterior sectors (90–180°) of the visual field of albino and pigmented rabbits. Differences in the optokinetic reflexes of albino and pigmented rabbits were not observed in the present experiment, probably because of the minimal stimulation of the anterior visual fields. The eye position of the nonstimulated eye was recorded. Vision of this eye was occluded with three layers of black cloth. In some experiments, the position of both the left and right eyes were recorded simultaneously during optokinetic and vestibular stimulation. In normal rabbits both the HVOR and HOKR are conjugate (18).

Behavioral testing

Optokinetic reflexes (OKR) and vestibuloocular reflexes (VOR) were measured before and after unilateral lesions of the left flocculus. The gain of the vestibuloocular reflex was determined from measurements of the peak eye velocities attained during each half cycle of sinusoidal rotation: $(V_R + V_L)/2V_T$, where V_R is peak compensatory eye velocity to the right; V_L is peak compensatory eye velocity to the left; and V_T is peak table velocity. At stimulation frequencies <0.02 Hz, two cycles of stimulation were usually measured. At frequencies >0.04 Hz, three or more cycles were usually measured.

Optokinetic reflex gains were measured using constant velocity, unidirectional monocular stimulation (G = peak eye velocity/stimulus velocity). For both vestibuloocular and optokinetic reflex testing, stimulation was delivered in an ascending order of frequencies or constant velocities. In preliminary tests of the VOR and OKR we observed that random switching between high and low frequencies caused a disruption in the behavioral state of the rabbits, and this required a longer measurement interval for each frequency of stimulation. Consequently, we adopted the method of ascending order of stimulus frequency and velocity that allowed a more rapid collection of data under more uniform behavioral conditions. All data were recorded with an FM tape recorder and subsequently measured and photographed from a storage oscilloscope.

Flocculus lesions

Lesions of the flocculus were made in rabbits that were anesthetized with either halothane or pentobarbital sodium (30 mg/kg). These lesions were made using three different surgical approaches (Fig. 2). *1*) Dorsal approach: an opening was made in the dorsal lateral occipital bone overlying the paramedian and ansiform lobules of the cerebellum. A glass suction pipette with a 1-mm tip diameter was inserted dorsoventrally through the lateral margin of the partially retracted ansiform lobule into the flocculus that was removed by aspiration. This operation caused partial damage to the ansiform lobule. *2*) Caudal approach: the caudal and lateral aspect of the occipital bone overyling the paraflocculus was removed with a dental burr. A suction pipette was inserted through the paraflocculus in a caudolateral-to-anteromedial direction, passing through the annulus of the anterior semicircular canal and into the flocculus. The flocculus was aspirated and the pipette was withdrawn. This approach caused damage to the dorsal paraflocculus as well as the paraflocular stalk that connects to the brain stem through the annulus of the anterior semicircular canal. In some instances there was additional damage to the lateral cerebellar nucleus that in its most lateral extent protrudes into the paraflocular stalk. *3*) Lateral approach: following a pre-auricular incision, the middle ear was opened and the tympanic membrane, the malleus, and incus were removed. With the visual guidance provided by a 40× dissecting microscope, a 2-mm opening was made though the medial wall of the middle ear just rostral and superior to the ampulla of the anterior semicircular canal. A suction pipette was inserted through this opening directly into the flocculus, and the flocculus was aspirated. This operation, when performed correctly, caused minimal extrafloccular damage.

Histological controls

At the conclusion of the eye movement recording experiments, rabbits were given an overdose

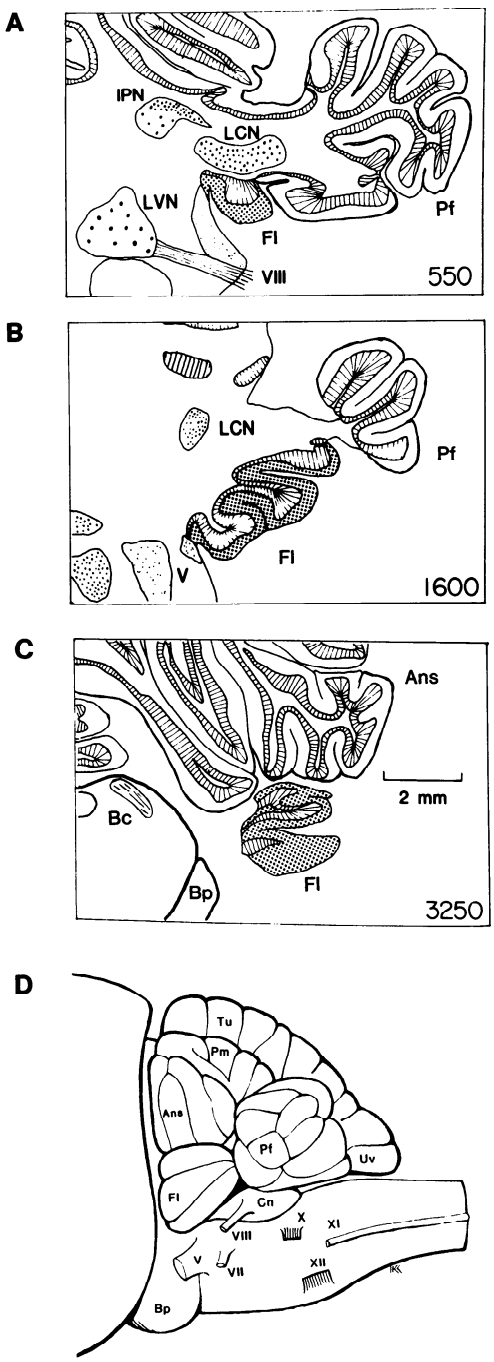

FIG. 2. Illustration of flocculus of rabbit. Cross sections of right flocculus are illustrated in caudal-rostral serial order, *A*, *B*, and *C*. Drawing of surface of rabbit cerebellum illustrates relative size of flocculus. Dorsal approach to flocculus damaged ansiform lobe and paramedian lobe. Caudal approach damaged part of paraflocculus and lateral cerebellar nucleus. Lateral approach through middle ear avoided major damage to non-

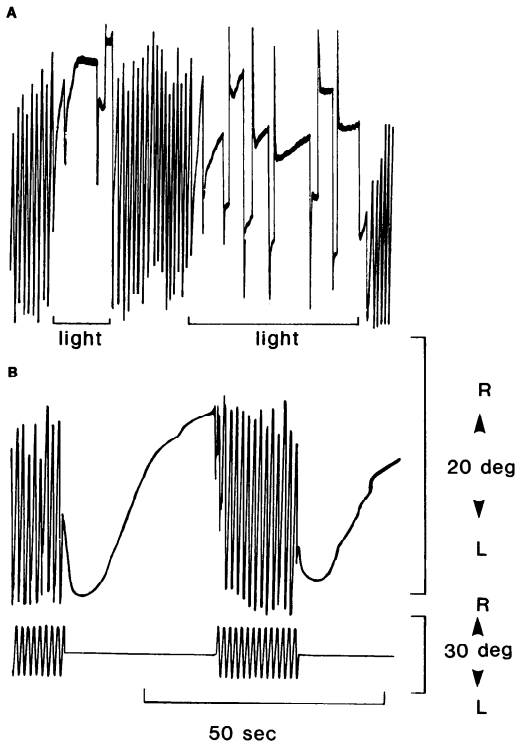

FIG. 3. Influence of 2 different left floccular lesions on spontaneous and vestibularly evoked eye movements. *A*: spontaneous nystagmus was observed 1 h after lesion was made (F #1) with dorsal approach. Nystagmus could be suppressed by exposing left eye of rabbit to dimly illuminated laboratory environment. *B*: 8 h following a left floccular lesion made with lateral approach (F #17), eyes drifted to right spontaneously, but drift was masked during vestibular stimulation at relatively high frequency, 0.8 Hz.

of pentobarbital sodium and perfused with a 15% formalin solution. The brain was removed and embedded in paraffin. Serial sections 15-μm thick were made through the regions of the dorsal cap of the inferior olive and through the cerebellum. Each section was mounted and stained with luxol fast blue and cresyl fast violet. The location and extent of the cerebellar lesions, as well as damage to extracerebellar structures were plotted onto a schematic cross-sectional representation of the

floccular structures. Fl, flocculus; Pf, paraflocculus; Cn, cochlear nucleus; Pm, paramedian lobe; Tu, tuber vermis; Uv, uvula; Ans, ansiform lobe; V, trigeminal nerve and nucleus; VII, facial nerve; VIII, auditory vestibular nerve; X, vagus nerve; XI, spinal accessory nucleus; XII, hypoglossal nerve; Bp, brachium pontis; Bc, brachium conjunctivum; IPN, interpositus nucleus; LCN, lateral cerebellar nucleus; and LVN, lateral vestibular nucleus.

cerebellum (Fig. 2, *A–C*). Early during this investigation it was observed that lesions of the flocculus cause retrograde cell loss in the contralateral dorsal cap of the inferior olive. Therefore an additional quantitative estimate of floccular damage was obtained from olivary cell counts made in the region of the dorsal cap. The cells in both the left and right dorsal caps were counted. A cell was positively identified as a dorsal cap neuron if it had a diameter of at least 10 μm and a distinct nucleus. No correction was made for double counting. However, because the same counting procedure was used throughout, the counts give a consistent estimate of the retrograde cell loss caused in the right dorsal cap by lesions of the left flocculus.

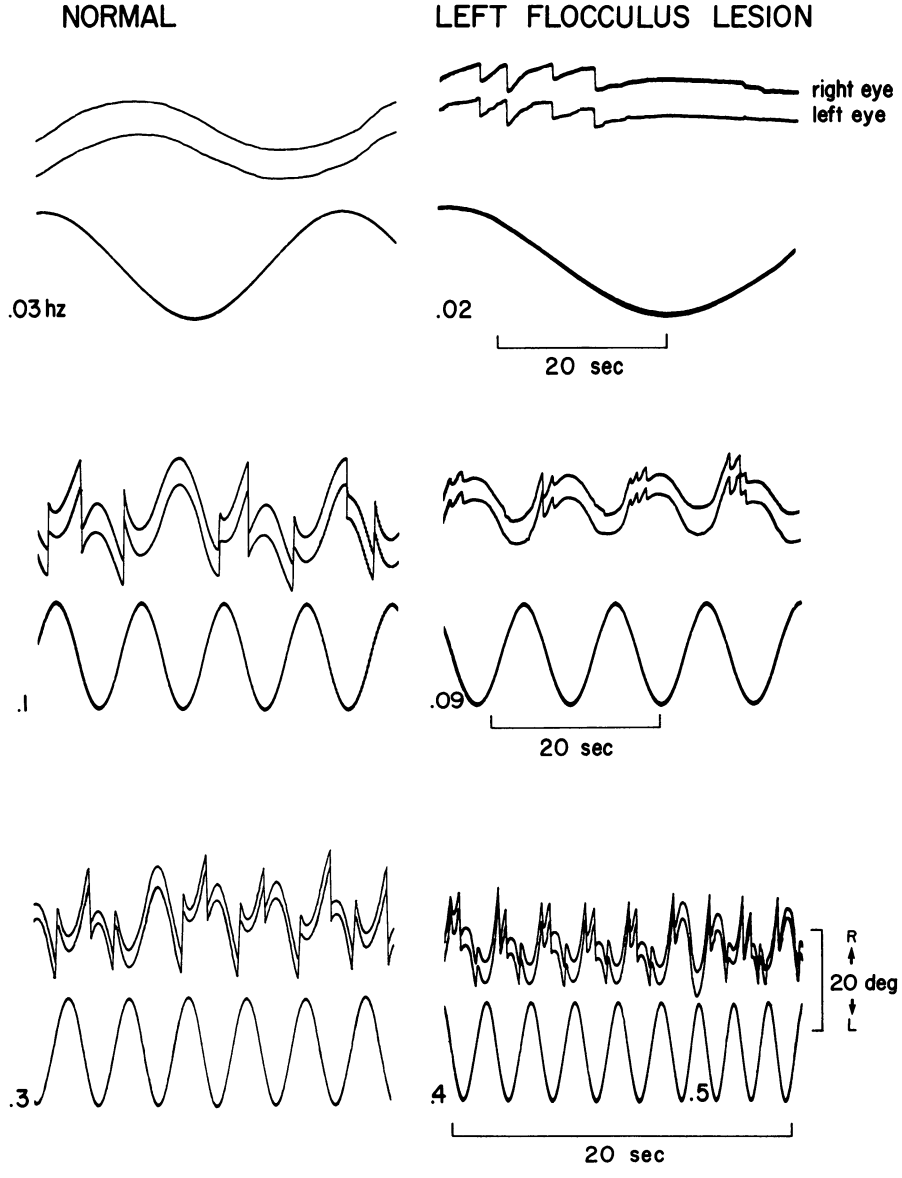

FIG. 4. Effects of lesion of left flocculus (caudal approach, F #7) on horizontal vestibuloocular reflex. Representative conjugate eye movement records are illustrated. Records were vertically displaced for illustrative purposes. Each series of traces, from top to bottom, consists of right eye position, left eye position, and table position. Lesions did not disrupt conjugacy of HVOR, but did cause a pronounced rightward velocity bias that was more evident at lower stimulus frequencies.

RESULTS

Spontaneous eye movements as a consequence of unilateral flocculus lesions

The immediate effect, observed within 15 min following placement of a unilateral left floccular lesion, was a conjugate horizontal spontaneous nystagmus with the slow phase toward the right side (opposite to the side of the lesion) when the animal was placed in total darkness. This spontaneous nystagmus lasted from several hours to two days depending on the extent of other cerebellar structures damaged. The nystagmus could be almost totally suppressed by permitting vision with either the left or the right eyes (Fig. 3*A*). With incomplete destruction of the flocculus there were also vertical and torsional components to the predominantly horizontal nystagmus. However, these components were more variable and were not systematically studied. The spontaneous horizontal nystagmus was diminished in velocity or reversed in sign if the subjacent vestibular nerve was damaged by the surgical operation.

These data emphasize the importance of maintaining secondary vestibular neurons functionally intact for the full expression of the spontaneous nystagmus following unilateral flocculectomy. To further study this relationship, unilateral floccular lesions were made in three rabbits that had been bilaterally labyrinthectomized one month ago. In none of these three rabbits was a spontaneous nystagmus evoked following left unilateral flocculectomies. These data imply that the spontaneous nystagmus may be attributed to a release of the vestibular nuclei from floccular inhibition.

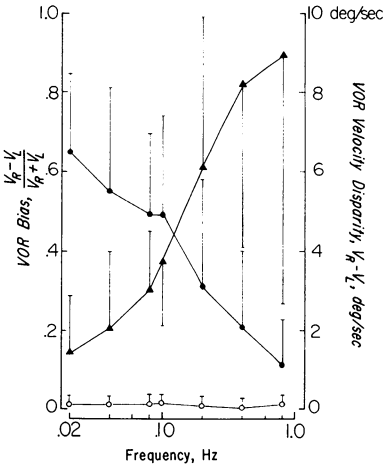

FIG. 5. Influence of left floccular lesions on HVOR velocity bias and velocity disparity. HVOR velocity bias $(V_R - V_L)/(V_R + V_L)$ and velocity disparity $(V_R - V_L)$ were measured in 10 rabbits before and within 4 days after lesions were made in left flocculus. Preoperative velocity bias (○); postoperative velocity bias (●); postoperative velocity disparity (▲). *Error bars* indicate 1 SD.

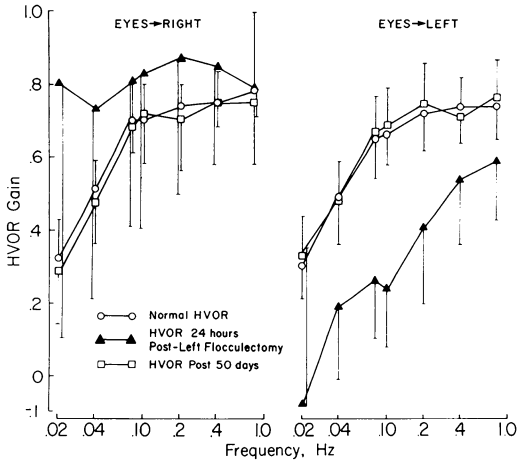

FIG. 6. Immediate and long-term effects of lesions of left flocculus on gain of HVOR. HVOR of 4 rabbits was tested before (○), 24 h after left flocculectomy (▲), and 50 days after left flocculectomy (□). Gain of HVOR was measured for each direction of eye movement in 4 rabbits. *Error bars* indicate 1 SD.

HVOR following floccular lesions

Unilateral left floccular lesions caused a conjugate rightward bias of eye movements evoked during horizontal vestibular stimulation (Fig. 4). This rightward bias of the eyes was frequency dependent. The absolute velocity of the bias, $V_R - V_L$, increased with increasing stimulus frequency, but the bias expressed as a ratio of the evoked eye velocity $V_R - V_L/V_R + V_L$, decreased (Fig. 5). These data indicate that the velocity of the spontaneous nystagmus caused by unilateral lesions was influenced by vestibular stimulation.

In a group of five rabbits, changes in the directional gains, (G_R, G_L) of the HVOR were measured before, immediately after, and 50 days after left flocculectomies were performed (Fig. 6). When measured within 24 h following the operation, G_R appeared to be elevated with respect to the preoperative

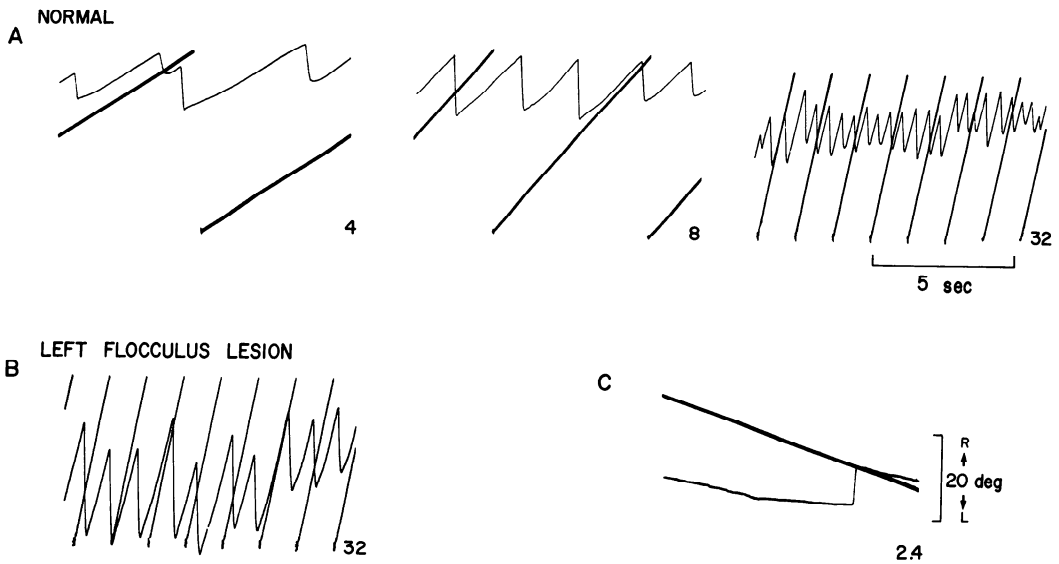

FIG. 7. Influence of unilateral lesion of left flocculus (caudal approach, F #7) on eye movements evoked by unidirectional constant velocity, monocularly viewed optokinetic stimuli. *A*: preoperative eye movements were recorded from right eye while left eye viewed a target moving in posteroanterior direction at velocities of 4, 8, and 32°/s. *B*: 7 days postoperatively rabbit could still follow high-velocity optokinetic targets viewed with left eye, but could not follow optokinetic targets viewed with right eye, *C*.

G_R, and G_L appeared to be decreased relative to the preoperative G_L at all frequencies tested. These effects could be attributed to rightward bias of eye movements caused by the left flocculus lesion. When the HVOR was measured 50 days later, G_R equaled the preoperative G_R, and G_L equaled the preoperative G_L.

Optokinetically evoked eye movements following unilateral floccular lesions

Constant velocity, monocular, unidirectional (posteroanterior) optokinetic stimulation was used to evoke optokinetic reflex eye movements before and after lesions were made to the left flocculus. The velocity of stimulus was slowly incremented after eye movements evoked at a previous stimulus velocity attained a constant value. As in previous experiments (8, 18) at low-stimulus velocities the gain of the optokinetically evoked eye movements approached unity and at higher stimulus velocities the gain decreased (Fig. 7*A*). Following left floccular lesions, the gain of eye movements evoked by optokinetic stimulation in the posteroanterior direction of the left eye (the eye ipsi-

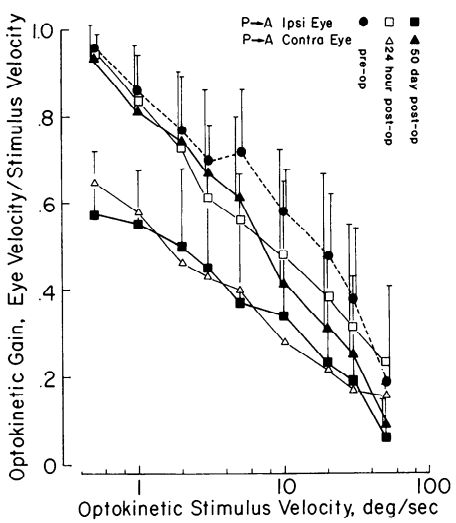

FIG. 8. Immediate and long-term influences of left floccular lesions on optokinetic reflex gain. Normal optokinetic reflex gain was measured in 5 animals before (●), 24 h (△ and □) and 50 days (▲ and ■) after lesions were made in left flocculus. Reflexes were measured for optokinetic stimulation in posteroanterior direction, viewed with eye ipsilateral (*squares*) and contralateral (*triangles*) to lesion. *Error bars* indicate 1 SD. 24 h postoperative SDs were of comparable magnitude, but were deleted for illustrative clarity.

lateral to the left floccular lesion) appeared almost normal when tested within 24 h following the left flocculectomy. The optokinetic gain of eye movements evoked by posteroanterior stimulation of the right eye (the eye contralateral to the lesioned left flocculus) apparently attenuated when measured within 24 h following the left flocculus lesion (Figs. 7*C* and 8). In other words, optokinetic stimulation of the eye that projected to the damaged flocculus appeared to evoke eye movements of nearly normal gain, and optokinetic stimulation of the eye that projected to the intact flocculus appeared to have a lowered gain. These results can be attributed to the bias of eyes to move toward the right following lesions of the left flocculus. It might seem appropriate to "correct" the optokinetic reflex

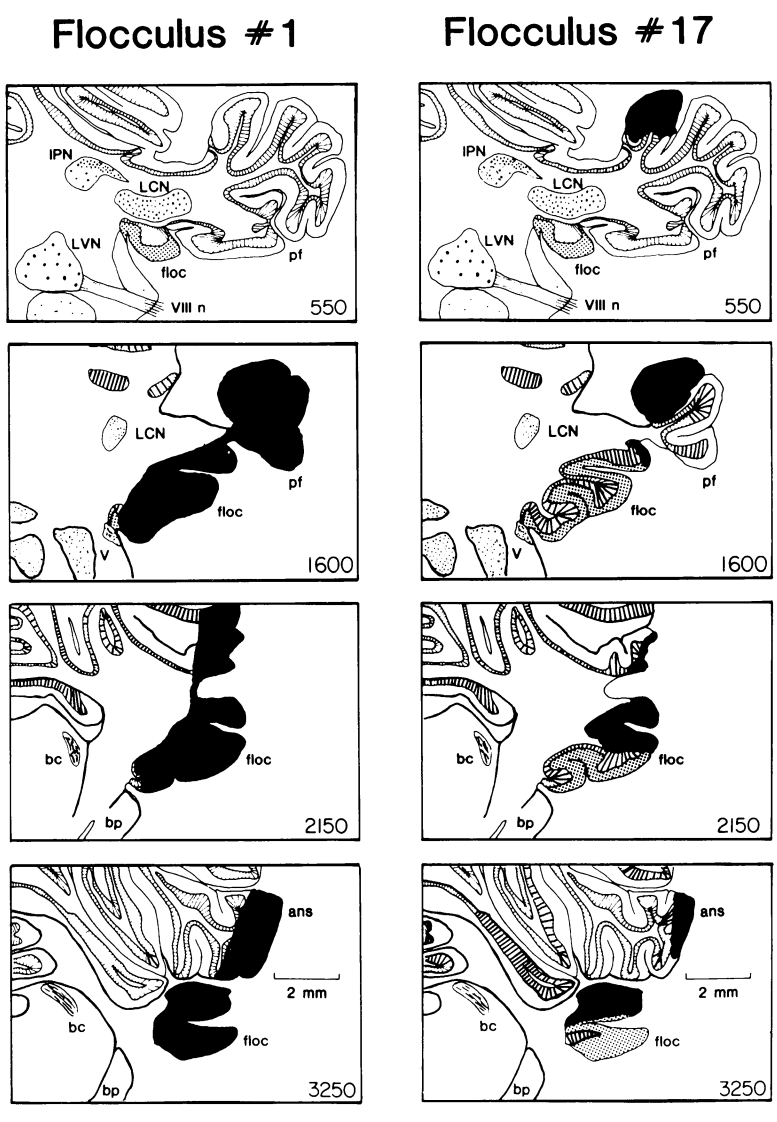

FIG. 9. Illustration of largest and smallest lesions of left flocculus. Flocculus #1 illustrates posteroanterior extent of a lesion made with a dorsal approach that caused considerable damage to ansiform lobe and some damage to caudal paraflocculus. Flocculus #17 illustrates a lesion made with a lateral approach. Minor damage to caudal paraflocculus and lateral ansiform lobe was caused. IPN, interpositus nucleus; LCN, lateral cerebellar nucleus; floc, flocculus; pf, paraflocculus; bc, brachium conjunctivum; bp, brachium pontis; V, trigeminal nucleus; VIII; auditory vestibular nerve; and ans, ansiform lobe.

gains by subtracting from the optokinetic velocity the slow-phase velocity of the spontaneous nystagmus recorded in the dark. However, previously it was shown that the velocity of vestibularly evoked eye movements appears to reflect an interaction of vestibular stimulation with the spontaneous drift (Fig. 5). There is no reason to suppose that optokinetic stimulation might not also interact with slow-phase velocity of the spontaneous nystagmus.

The long-term consequences of lesions of the left flocculus on optokinetic reflex gains could be assessed accurately only after the disappearance of the short-term rightward bias of eye movements. Fifty days following the left flocculectomy, when the HVOR gains (G_R, G_L) were symmetrical and there was no spontaneous nystagmus in the dark, the gain of the HOKR for eye movements evoked by posteroanterior stimulation of the eye ipsilateral to the lesion was reduced and the gain of the HOKR for eye movements evoked by posteroanterior stimulation of the eye contralateral to the left flocculus lesion was near normal (Fig. 8). Thus the permanent consequence of a unilateral lesion of the left flocculus was a reduced HOKR gain for eye movements evoked by posteroanterior optokinetic stimulation of the left eye.

Histological evaluation of floccular lesions

The extent and the size of the floccular lesions were estimated from serial histological reconstructions through the cerebellum and brain stem areas adjacent to the flocculus. Damage to the flocculus was graded on a 5-point scale; a grade of 5 corresponding to total destruction of the flocculus and a grade of 1 corresponding to <20% damage to the flocculus. Representative large and small lesions are illustrated in Fig. 9. The lesion in flocculus #1 was made with the dorsal ap-

TABLE 1. *Quantitative estimates of the size of unilateral floccular lesions*

Rabbit	Surgical Approach	Lesion Size	Extraflocculor Damage	Survival, days	Posterior DC (850 μm) L	Posterior DC (850 μm) R	Total DC (2,000 μm) L	Total DC (2,000 μm) R
F1	Dorsal	4	ans, pf	0				
F2	Dorsal	5	ans, pm	0				
F3	Dorsal	4	al, pm	10				
F4	Dorsal	5	ans, pf	4	1,162	742	2,952	2,130
F5	Dorsal	5	ans, pf, lcn	21	1,484	529	3,792	2,038
F6	Dorsal	3	ans, pf, lcn	7	1,468	668	3,776	1,696
F12	Dorsal	2	ans	11	1,386	1,146	4,496	3,946
F15	Dorsal	5	ans, pf, lcn	2	1,271	1,175	3,434	3,256
F7	Caudal	5	pf, lcn	30	822	336	2,358	1,219
F8	Caudal	5	pf	4	1,096	414	2,948	1,346
F9	Caudal	2	pf	39	1,306	1,188	3,478	3,250
F10	Caudal	5	pf, lcn	126	1,252	450	3,768	1,766
F11	Caudal	4	pf, lcn	107	1,256	704	3,602	2,034
F13	Caudal	5	pf, lcn	100	920	365	3,210	1,635
F14	Lateral	5	ans, lcn	11	1,216	628	3,040	1,826
F16	Lateral	5	scp	17	1,182	350	3,172	1,470
F17	Lateral	2	pf	132	1,232	908	3,216	2,450
F18	Lateral	3	ans	127	1,358	764	3,142	2,288
F19	Lateral	5	pf	87	1,210	590	3,376	1,516
F20	Lateral	4	lcn	12	1,208	405	3,426	1,214
F21	Lateral	3		13				

Direct estimates of lesion size were based on measurements of serially examined cross-sectional areas. A grade of 1 corresponds to 20% damage; a grade of 5, 100%. Extraflocculor areas that sustained damage are also listed. ans, Ansiform lobe; pf, paraflocculus; pm, paramedian lobe; lcn, lateral cerebellar nucleus; and scp, superior cerebellar peduncle.

proach and received a grade of 4. The lesion in flocculus #17 was made with the lateral approach and received a grade of 2 (Fig. 9 and Table 1). In addition, damage to extraflocculus areas was noted and listed in Table 1. Floccular lesions made from a dorsal approach always caused considerable damage to the ansiform lobe and modest damage to the paraflocculus and to the lateral cerebellar nucleus. Floccular lesions made through the paraflocculus always destroyed most of the paraflocculus and some of the lateral cerebellar nucleus. Floccular lesions made from a lateral approach through the middle ear caused some damage to the ansiform lobe, the superior cerebellar peduncle, and the paraflocculus. However, the damage to these areas made during lesions of the flocculus with a lateral approach was small relative to the damage caused to extraflocculus structures made with the caudal and dorsal approaches.

An anatomical analysis of the brain stems of floccular lesioned rabbits revealed that the unilateral lesions caused degeneration of neurons in the contralateral dorsal cap of the inferior olive. This cell loss accounted for as much as 65% of the dorsal cap neuronal population. Cell loss in the dorsal cap was evident in animals sacrificed more than two days after the floccular lesion was made and the degeneration appeared to be complete within seven days. This retrograde cell loss was estimated by counting the cells in the region of the dorsal cap bilaterally in every other section of serially sectioned brain stems of the floccular-lesioned animals. The total neuronal dorsal cap population was estimated from these cell counts. The cell counts were broken down into two anteroposterior regions; one area included the caudalmost 850 μm of the dorsal cap. This area comprised the region of the dorsal cap that contains neurons sensitive to posteroanterior optokinetic stimulation of the contralateral eye. The second area included the entire dorsal cap, extending 2 mm from the caudal pole to the rostralmost extent defined by the ventrolateral outgrowth (Fig. 10). An estimate of floccular damage made from the extent of retrograde cell loss was compared with the estimate of the floccular damage made by direct measurement of the lesion size. The percentage of cell loss in the posterior dorsal cap was correlated with floccular lesion size, $r = 0.69$ for the posterodorsal cap, and $r = 0.65$ for the total dorsal cap. Examples of the retrograde cell loss at the level of the dorsal cap caused by damage to the left flocculus are illustrated in Figs. 10 and 11.

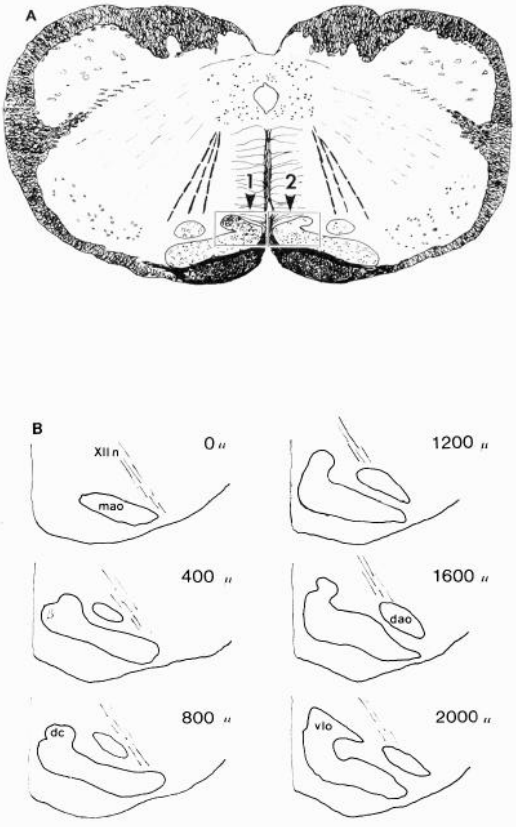

FIG. 10. Brain stem of the rabbit illustrating regions of inferior olive where cellular degeneration was measured following lesions of left flocculus. *A*: area of inferior olive delineated by boxes 1 and 2 is illustrated in higher-power photomicrographs in Fig. 11. *B*: posteroanterior extent and configuration of dorsal cap is illustrated. XIIn, hypoglossal nerve; mao, medial accessory olive; dao, dorsal accessory olive; vlo, ventrolateral outgrowth; β, beta nucleus; and dc, dorsal cap of Kooy.

An attempt was made to determine if the velocity of the spontaneous nystagmus caused by floccular lesions was correlated with the size of the lesion. These correlations were $r = 0.33$ for direct measurements of floccular damage and $r = 0.29$ for indirect measurements of floccular damage (retrograde cell loss in the posterodorsal cap).

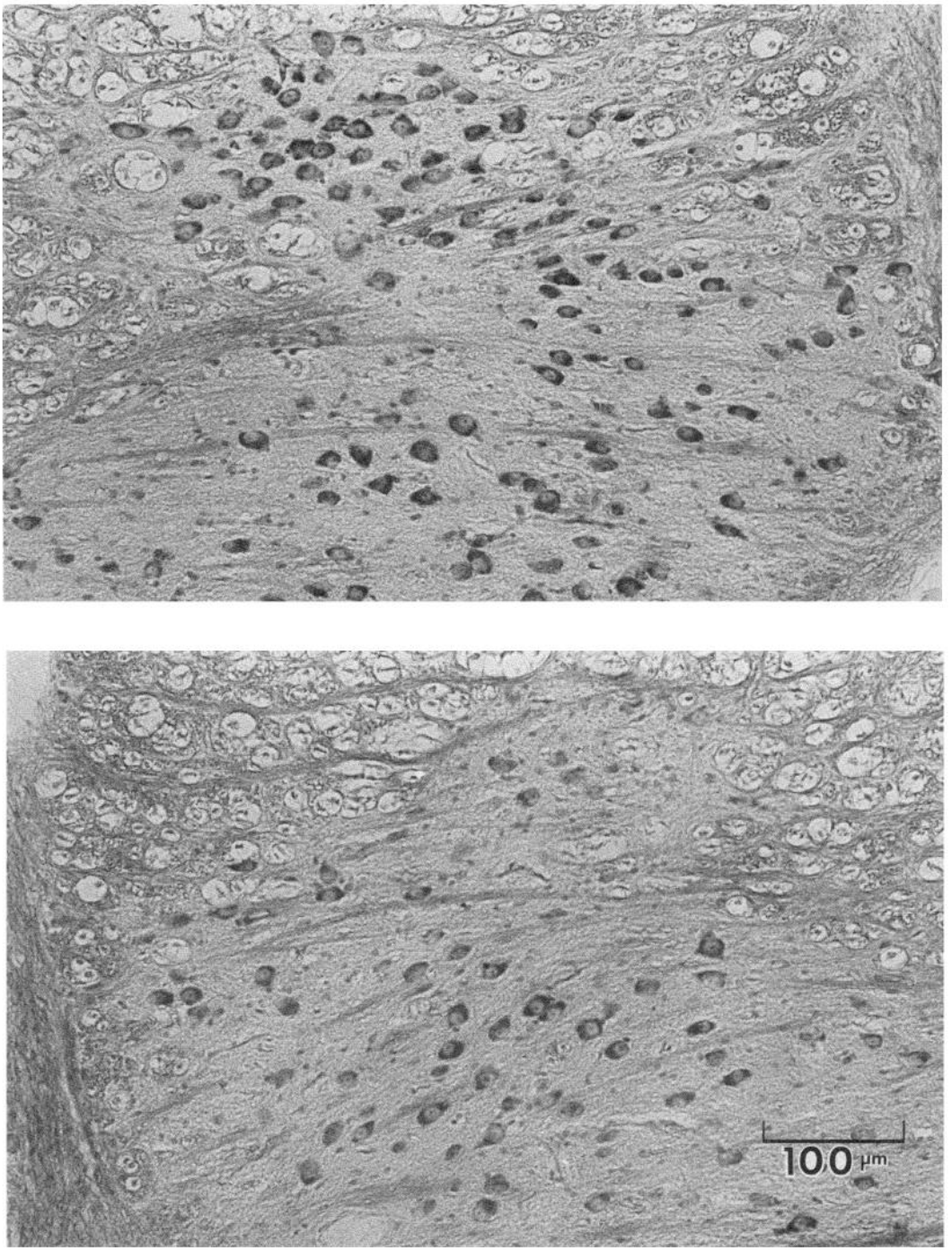

FIG. 11. Photomicrograph of left (*top*) and right (*bottom*) caudal dorsal caps of F #7; sacrificed 30 days following a left floccular lesion. Note that right dorsal cap has fewer neurons than does left dorsal cap, due to degeneration caused by left floccular lesion. Brain stem location of these photomicrographs is illustrated in Fig. 10*A*.

DISCUSSION

Evaluation of behavioral deficit caused by unilateral flocculus lesions

There were four principal behavioral observations made on rabbits that received unilateral lesions of the flocculus: *1*) immediately after the left flocculus lesions were made, a spontaneous, low-velocity conjugate nystagmus with a rightward directed slow phase ensued in the dark. *2*) The HVOR was biased toward the right, but the average gain of the HVOR, $[(G_R + G_L)/2]$, was not modified. *3*) The HOKR gain for posteroanterior stimulation of the left eye, the eye which would project to the damaged flocculus through the contralateral dorsal cap of the inferior olive, appeared normal immediately postoperatively. Posteroanterior stimulation of the right eye, the eye which would project to the intact flocculus, resulted in a reduced HOKR gain. *4*) Fifty days following the unilateral lesion there was no spontaneous nystagmus and the gain of the HVOR was normal and without bias. There was a permanent reduction in the gain of the HOKR for posteroanterior stimulation of the left eye.

Recent experiments on the fine topography of both the dorsal cap of the inferior olive (40) and the flocculus (26, 27, 38) suggest that directionally specific visual information in both the horizontal and vertical planes is encoded in separate locations of the inferior olive and the flocculus. This functional separation of vertical and horizontal pathways is also maintained by cerebello-vestibulo-oculomotor projections, as determined by electrical stimulation of the flocculus (26, 27). Although a few of the rabbits in the present experiment evinced a vertical component associated with the spontaneous horizontal nystagmus following unilateral flocculectomy, this vertical component was small. It is possible that both the upward eye movement and downward eye movement regions were consistently damaged by our relatively large lesions, thereby decreasing the effect that destruction of either the upward or downward area alone would produce.

Anatomical measurements of floccular damage

Three different surgical approaches to the flocculus produced essentially equivalent behavioral deficits. The conjugate nystagmus (slow phase to the right) caused by lesions of the left flocculus was opposite to the nystagmus that would be produced by damage to the subjacent vestibular nerve or vestibular nuclei. If the left vestibular nerve were damaged, a nystagmus would be produced with a leftward slow-phase velocity. The polarity of the spontaneous nystagmus is of importance because the vestibular nerve lies ventral and posterior to the flocculus and could easily be damaged during surgery by direct insult or by subsequent indirect and temporary injury caused by depolarization from excessive extracellular concentration of potassium due to hemorrhaging or by compression caused by swelling of the cerebellum and brain stem. In fact such indirect damage to the vestibular nerve may mask the behavioral evidence of damage to the flocculus. In two preliminary experiments we observed a leftward slow-phase spontaneous nystagmus when our lesion damaged both the left flocculus and the left vestibular nerve. These data emphasize the importance of preserving the spontaneous activity of vestibular primary afferent input to the vestibular nuclei for the expression of the effect of floccular lesions. This point is complemented by our failure to observe the rightward spontaneous nystagmus following unilateral left flocculectomies in three rabbits that previously had been bilaterally labyrinthectomized. The data reported here are in general agreement with results obtained from other investigations. Floccular lesions produce only a temporary impairment of the HVOR in cats (13, 19) and monkeys (41–43, 47). Unilateral lesions of the flocculus cause a temporary contralateral drift of the eyes in both cats (13) and monkeys (47). These observations are in apparent conflict with a report based on data obtained from rabbits in which unilateral floccular lesions produced an ipsilateral drift of the eyes (30). It seems likely that the floccular lesions in this later report also damaged the vestibular nerve or nuclei. Furthermore, our observation that unilateral floccular lesions cause a conjugate bias of eye movements with no reduction in gain of the HVOR (e.g., Figs. 4 and 5) are in apparent conflict with previous reports in which it was suggested that unilateral lesions of the flocculus only influence the ipsilateral eye and permanently reduce the gain of the ipsilateral

eye during horizontal vestibular stimulation (24, 28). Our observations are based on eye movement recordings obtained simultaneously from both the left and right eyes, pre- and postoperatively. We think that these controls are essential in order to evaluate the conjugacy of eye movements following unilateral floccular lesions.

The possibility that transient damage to the underlying vestibular nerve may mask the full expression of the behavioral deficit caused by a unilateral lesion of the flocculus may account in part for the relatively low correlation, $r = 0.33$, between the extent of floccular damage and the velocity of the spontaneous nystagmus. This relatively low correlation might also be attributed, in part, to a lack of variation in the size of the floccular lesion. Most of the surgical lesions destroyed 80% or more of the flocculus (see Table 1). Hence statistically our observations were compressed to a narrow range of lesion sizes.

Virtually all previous behavioral investigations of the effects of floccular lesions have noted impaired visual suppression of vestibular nystagmus or reduced gains of optokinetic reflexes (13, 30, 41–44, 47). The present experiment clearly demonstrates that the permanent effect of floccular damage is a reduction in the gain of the HOKR. The visual olivocerebellar projection from the dorsal cap may provide the afferent link in this floccular circuit, although at present one cannot rule out the possibility that mossy fiber pathways may also contribute. These data imply that the flocculus may provide a mechanism by which low-velocity retinal slip information caused by eye or head movement may supersede the relatively higher-frequency information concerning head movement arising from the semicircular canals.

Relationship of behavioral deficit to olivocerebellar circuitry

The behavioral deficit produced by a unilateral floccular lesion can be understood in terms of the diagram depicted in Fig. 1. Destruction of the left flocculus removes a descending inhibitory influence onto the left vestibular nuclei, particularly the left medial vestibular nucleus. This removal of inhibition would be functionally equivalent to exciting the left medial vestibular nucleus by head angular acceleration to the left. This leftward rotation evokes rightward eye movements. Conversely damage to the left vestibular nerve or left vestibular nuclei would cause a reduction in the activity of the left vestibular nuclei and evoke ipsilaterally directed leftward conjugate eye movements.

Lesions of the left flocculus can be interpreted as the most extreme form of inhibition of Purkinje cell function and the most extreme form of Purkinje cell disinhibition of the vestibular nuclei, with a consequent imbalance in the activity of the vestibular nuclei. This imbalance of activity leads to a spontaneous nystagmus with the slow phase to the right, a bias of the HVOR to the right, and a bias of the HOKR to the right. This rightward bias temporarily obscures the primary deficit; a reduced gain of the HOKR that is caused by the absence of the CF-mediated optokinetic modulation of floccular Purkinje cell activity. Presumably within a few days balance is restored between the vestibular nuclei, and it is at this time that the absence of visual CF-mediated reduction of SS activity of the left flocculus is expressed as a permanent reduction in the gain of the HOKR.

Floccular control of vestibular pathways and antiphasic behavior of CFRs and SSs

If the flocculus provides the anatomical substrate by which sensory information of low temporal frequency (retinal slip of low velocity) supersedes information of relatively high temporal frequency (angular acceleration of the head), how is this transition accomplished? Perhaps in two stages. Angular head displacement would provide feedback from both the semicircular canals and the visual olivocerebellar pathway. Leftward angular acceleration would stimulate the left horizontal semicircular canal that would transiently excite secondary neurons in the left vestibular nucleus. Posteroanterior optokinetic stimulation of the left eye caused by leftward head movement would activate the visual olivocerebellar pathway originating from the right dorsal cap (Fig. 1). This optokinetically evoked CF activity has both a transient component and a steady-state component associated with posteroanterior retinal slip velocity (6, 39). The optokinetically evoked CF discharge could cause a transient increase in CFRs of Purkinje cells, lasting 10–100 ms, followed by a net reduction in SSs lasting seconds (8). This biphasic change in Purkinje cell activity would inhibit and then disinhibit

the activity of secondary neurons in the vestibular nucleus. In other words the visual olivocerebellar input could exert a biphasic influence on the vestibular nucleus. The first component of this biphasic influence, the transient inhibition of secondary vestibular neurons, would be the opposite sign of the primary afferent vestibular transient caused by leftward head acceleration. This transient cancellation would be followed by a slow, visually controlled disinhibition of the vestibular nucleus. The antiphasic behavior of CFRs and SSs could be viewed as a mechanism by which the flocculus cancels higher frequency vestibular information, but is synergistic with lower frequency vestibularly mediated activity. In a more general sense CF-mediated activity might provide a lower temporal frequency spatial reference used for postural control. In addition to CF-encoded retinal slip of low velocity other low temporal frequency sensory events such as otolithic vestibular inputs and neck proprioceptive inputs might also be preferentially encoded by CF-cerebellar pathways. Although our data imply how CF-mediated optokinetic stimulation may influence eye movements, they do not provide an appropriate explanation for how independently mediated vestibular and visual mossy fiber activity might contribute to eye movements.

The principal finding of the present experiment is that damage to the flocculus permanently impairs the gain of the optokinetic reflex. These data do not exclude the possibility that the flocculus may participate in longer-term modifications of vestibular, optokinetic, and perhaps other postural reflexes. However, the present data suggest that experiments that purport to demonstrate the role of the flocculus in such long-term modifications must distinguish between the use of retinal slip velocity information to augment optokinetic reflexes and the use of this information to modify eye movements. Obviously if the gain of the HOKR is impaired following a unilateral flocculectomy, the ability to modify vestibuloocular reflexes with optokinetic information would also be impaired (25, 28, 37). However, the demonstration of an inability to modify eye movements would not provide decisive evidence for or against the idea that the visual modification of vestibuloocular reflexes occurs at the level of the flocculus.

ACKNOWLEDGMENTS

This research was supported by National Institutes of Health Grant EY-04778, the Oregon Lions Sight and Hearing Foundation, and the Consiglio Nazionale delle Ricerche of Italy.

Received 23 April 1984; accepted in final form 16 August 1984.

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