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Eye Movement Evoked by Stimulation of Purkinje Cell Zones of the Cerebellar Flocculus in the Cat

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Summary. **Anatomically, the cat cerebellar flocculus has been divided into three Purkinje cell zones (caudal, middle and rostral) on the basis of differential efferent projections to the vestibular nuclei.[1,2] In the present study, eye movement evoked by microstimulation of the flocculus was investigated by a video sysytem in ketamine-anesthetized cats. Stimulation of the rostral or caudal zones on the right side evoked a downward movement of both eyes combined with the intorsion of the left eye and a very small amplitude of extorsion of the right eye (downward-rotatory type). Stimulation of the middle zone evoked the abduction of the right eye and adduction of the left eye (horizontal type). Stimulation in the border areas between the zones evoked a combined movement of the downward-rotatory and horizontal types. Extorsion of the right eye and intorsion of the left eye were evoked from the border area between the rostral zone and the cerebellar peduncle. An upward movement of both eyes or a combined upward movement and contralateral horizontal movement of both eyes were found in the border area between the rostral zone and the eighth nerve. Anatomically defined rostral and caudal zones may be related to eye movement control on the vertical plane. The middle zone may be related to eye movement control on the horizontal plane.**

INTRODUCTION

The cerebellar flocculus of the cat has been divided into three Purkinje cell zones (caudal, middle, and rostral) on the basis of differential efferent projections: Purkinje cells in the caudal zone send their axons to the y-group of the vestibular nuclei and the subnucleus lateralis parvocellularis of the lateral cerebellar nucleus, those in the middle zone project to the medial vestibular nucleus, and those in the rostral zone project to the superior vestibular nucleus.[1,2] A subsequent study has suggested that the middle zone is responsible for eye movement control on the horizontal plane, and the rostral and caudal zones are responsible on the vertical plane (Sato and Kawasaki, in press). This suggestion was made on the basis of differential directions of eye movement as anticipated from the neuronal network of Purkinje cell zones of the cerebellar flocculus (Sato and Kawasaki, in press). However, the actual eye movement evoked by stimulation of the cat flocculus has not yet been fully investigated, except for one study which recorded an evoked movement of unilateral eye by electro-oculography.[3]

The present study was undertaken to investigate the rotatory, horizontal, and vertical components of movement of both eyes evoked by stimulation of the floccular Purkinje cell zone. Eye movement recordings were made through a video system composed of a video camera, television, videotape recorder, and video printer with frame memory and a monitor screen.

MATERIALS AND METHODS

In the present experiments, data were obtained from 11 cats (1.6-3.5 kg body weight). The cats were initially anesthetized with ketamine hydrochloride (50 mg/kg, i.m.), supplemented with continuous infusion of ketamine (5 mg/kg/h, i.v.). In addition, all open wounds were covered with 2% lidocain jelly. A partial craniectomy was performed to expose the cerebellar hemisphere on the right side.

For electrical stimulation, a glass-insulated tungsten electrode with a tip exposure of 20-30 μm in length and 10-15 μm in diameter was inserted stereotaxically into the flocculus on the right side. In each cat, 2-8 tracks were run in and around the flocculus. The stimulated sites along each track were 0.25 mm apart. Rectangular train pulses (pulse width 0.2 ms, pulse rate 200 Hz, intensity less than 0.1 mA, and train duration 1 sec) were passed through the electrode. Stimulus currents were measured as a voltage drop across a 1K ohm resistor placed between the electrode and a constant-current stimulator.

For the observation of eye movement, a white cross on black tape (Kroy 809, Kroy Inc.) was attached to the cornea on both sides of the cat's eye after local anesthesia. In addition, white threads were stretched in front of the cat's eyes in horizontal and vertical directions. The movement of both white crosses relative to the white threads was displayed through a video camera and television and recorded using a videotape recorder.

At the termination of each experiment, more than two sites in the flocculus were marked with electrical lesions by passing a cathodal current of 50 μA for 15 sec. The animal was deeply anesthetized with pentobarbital sodium and perfused first with normal saline and then with 10% formalin. The cerebellum was cut serially on a freezing microtome in sagittal sections of 100 μm, and was stained with 0.1% cresyl violet. Based on the locations of electrolytic lesions and stimulating-electrode tracks, the stimulated sites were reconstructed on enlarged drawings made with the aid of a camera lucida.

Off-line analysis of evoked eye movement was made by a monitor screen and a video printer with frame memory composed of two field memories. The eye position before stimulation was displayed on a monitor screen using first field memory, and that during stimulation was displayed using second field memory. The direction of the eye movement was investigated by alternative displays of the first and second field memories. The eye movements evoked by stimulation were demonstrated by simultaneously printing the first and second field memories.

RESULTS

Explorations by a stimulating microelectrode were performed in various parts of the flocculus. The type of evoked eye movement with a threshold at less than 50 μA were investigated for each stimulation site. Evoked eye movement was usually slow and smooth. With the increase of the stimulus current, slow and smooth eye movements tended to be interrupted by quick phases of eye movement in the opposite direction, resulting in nystagmus. The present experiment is concerned with the slow and smooth eye movement, and those data when the nystagmus was detected were discarded. The nystagmus was scarcely observed at stimulus intensities of less than 50 μA. Threshold currents for evoking slow and smooth eye movement were low (5-20 μA) at the stimulation sites located in the Purkinje cell layer or the white matter, while those in the granular and molecular layers were high (more than 20 μA).

Explorations in and around the caudal zone of the flocculus

Explorations were perfomed in and around the caudal zone of the flocculus[1,2] at a mediolateral level of 2.0-2.5 mm from the lateral edge of the flocculus (Fig. 1A). In the track including the "C" and "D" sites (Fig. 1B), two types of slow and smooth eye movement were found: (1) the downward-rotatory type (Fig. 1C), a downward movement of both eyes combined with intorsion of the left (contralateral to the stimulation side) eye; and (2) the combined downward-rotatory and horizontal type (Fig. 1D), the downward-rotatory type movement combined with abduction of the right eye and adduction of the left eye. It should be mentioned that the downward-rotatory type movement was usually accompanied by a very small amplitude of the extorsion of the right (ipsilateral to the stimulation side) eye, which was noted by an alternative display of the eye position before and during stimulation on a monitor screen. This rotatory movement was so small that we could not clearly demonstrate it on a video print. In the next rostral track, which penetrates the border area between the caudal and middle zones, the combined downward-rotatory and horizontal type was found (Fig. 1B). In this rostral track, the vertical and rotatory components were small and the horizontal component was large.

In another cat, the caudal zone of the flocculus was explored at mediolateral levels of 0.5-1.0 and 1.5-2.0 mm. It was confirmed that stimulation of the caudal zone evoked eye movement of the downward-rotatory type (not illustrated).

Explorations in and around the middle zone of the flocculus

Electrode-penetrations were made in and around the middle zone of the flocculus at a mediolateral level of 1.0-1.5 mm from the lateral edge of the flocculus (Fig. 2A). In the track including the "C" site in addition to

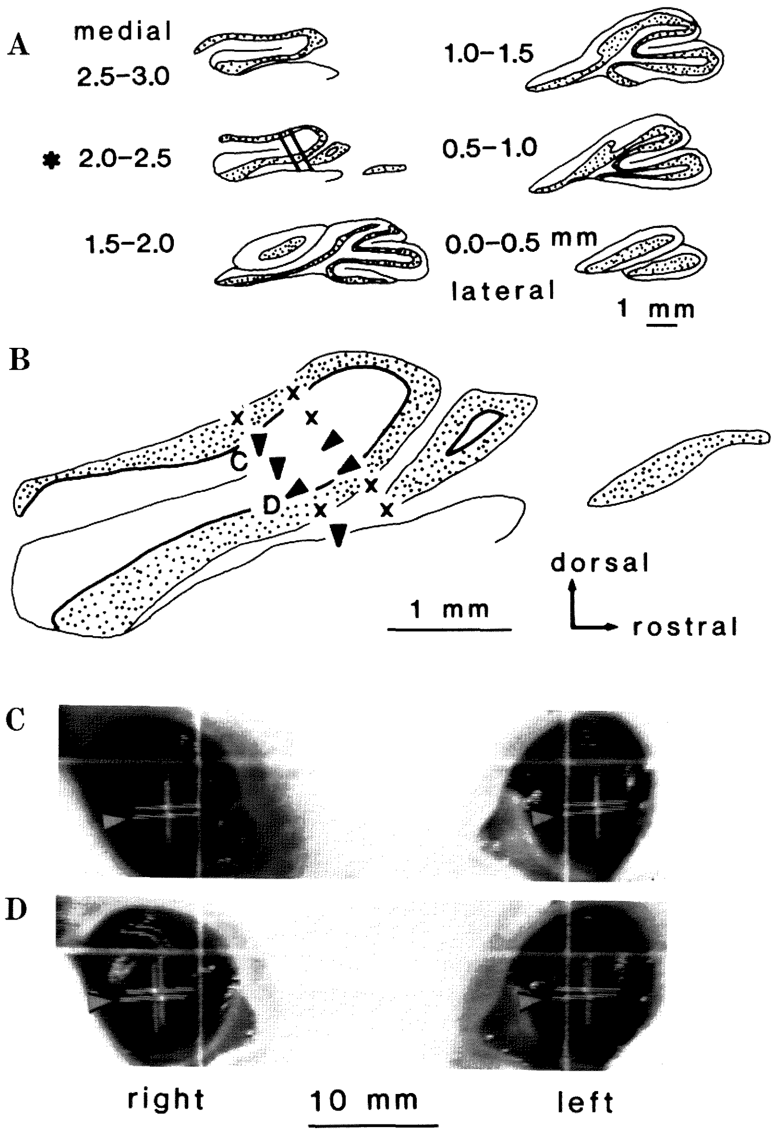

Fig. 1. Explorations in and around the caudal zone of the flocculus (A). Note that stimulation evoked eye movement of the downward-rotatory type (B, C) and the combined downward-rotatory and horizontal type (B, D). Downward and oblique triangles in B represent eye movement of the downward-rotatory type and combined downward-rotatory and horizontal type, respectively. White triangles in C and D show the position of white crosses during stimulation. A: line drawings of a 1 in 5 series of 100 μm sagittal sections through the right flocculus showing the location of electrode tracks (straight lines). B: higher magnification of the section with an asterisk in A showing the types of eye movements evoked by local stimulation with a threshold current of less than 50 μA. Thick lines represent the Purkinje cell layers. The molecular layers are adjacent to the Purkinje cell layers. Dotted areas represent the granular layers. The white matter is adjacent to the granular layers. X represents the site in which stimulation of less than 50 μA could not evoke eye movement. C: video print before and during stimulation of the "C" site in B showing the movement of white crosses attached to corneas of the cats' eyes. In our video system, it takes 1/30 sec for one frame. The one frame is composed of two fields, each of them taking 1/60 sec. The first field just before stimulation and the 60th field after the onset of stimulation were printed as one frame. D: video print before and during stimulation of the site "D" in B.

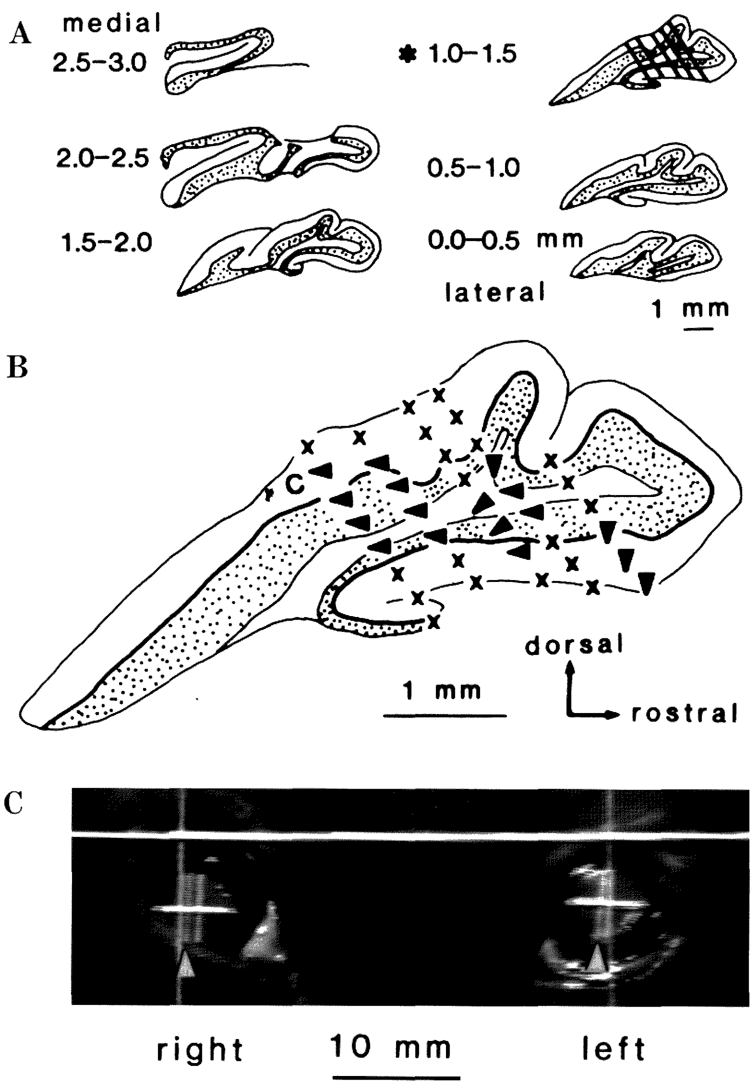

Fig. 2. Explorations in and around the middle zone of the flocculus (A). Note that stimulation evoked eye movement of the horizontal type (B, C). Horizontal, oblique, and downward triangles in B represent the eye movement of the horizontal, combined downward-rotatory and horizontal, and downward-rotatory types, respectively. White triangles in C show the position of white crosses during stimulation. See Fig. 1A, 1B, and 1C for captions of A, B, and C, respectively.

the next rostral track (Fig. 2B), the horizontal type (abduction of the right eye and adduction of the left eye) was discovered (Fig. 2C). The amplitude of the movement of the left eye (contralateral to the stimulation side) was 50-90% of that of the right eye. In the more rostral two tracks, the horizontal type was found near a Purkinje cell layer, and the horizontal, downward-rotatory, and combined downward-rotatory and horizontal types were found in the granular layer and the white matter (Fig. 2B). In the

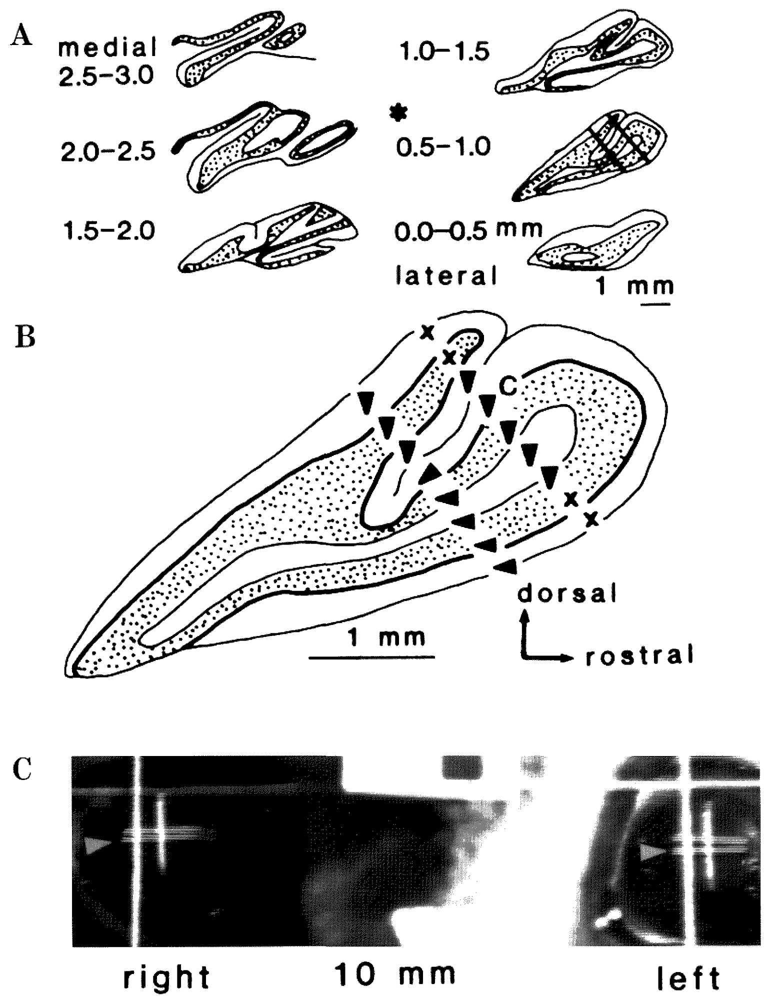

Fig. 3. Explorations in and around the rostral zone of the flocculus at a lateral level (A). Note that stimulation evoked eye movement of the downward-rotatory type (B, C). See Fig. 1 for symbols of black (B) and white (C) triangles. See Fig. 1A, 1B, and 1C for captions of A, B, and C, respectively.

most rostral track which penetrates the rostral zone, the downward-rotatory type was found in the Purkinje cell and molecular layers (Fig. 2B).

In another cat, the middle zone of the flocculus was explored at mediolateral levels of 0.5-1.0 and 1.5-2.0 mm. It was confirmed that stimulation of the middle zone evoked eye movement of the horizontal type (not illustrated).

Explorations in and around the rostral zone of the flocculus

Explorations were performed in and around the rostral zone of the flocculus at a mediolateral level of 0.5 - 1.0 mm from the lateral edge of the flocculus (Fig. 3A). In the track including the "C" site (Fig. 3B), the downward-rotatory type was found (Fig. 3C). In the next caudal track which penetrates the border

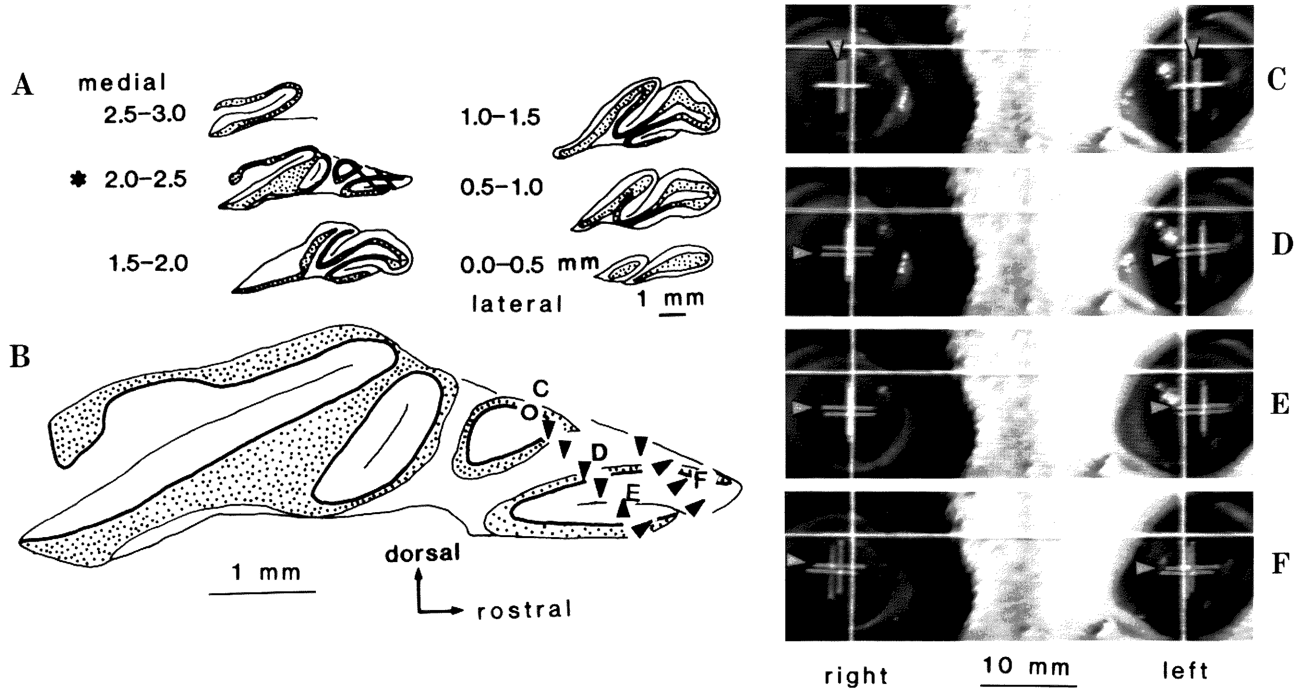

Fig. 4. Explorations in and around the rostral zone of the flocculus at a medial level (A). Note that stimulation evoked eye movement of the rotatory type (B, C) and the downward-rotatory type (B, D), the upward type (B, E), and the combined upward and horizontal type (B, F). The open circle, downward triangles, upward triangles, and oblique triangles in B represent the eye movement of the rotatory, downward-rotatory, upward, and combined upward and horizontal types, respectively. White triangles in C-F show the position of white crosses during stimulation. See Fig. 1A, 1B, 1C, and 1D for captions of A, B, C, and D, respectively. E: video print before and during stimulation of the "E" site in B. F: video print before and during stimulation of the "F" site in B.

area between the rostral and middle zones, the downward-rotatory, combined downward-rotatory and horizontal, and horizontal types were found (Fig. 3B).

Explorations were made in and around the rostral zone at a mediolateral level of 2.0-2.5 mm (Fig. 4A). In the "C" site (Fig. 4B), the rotatory type (extorsion of the right eye and intorsion of the left eye) was discovered (Fig. 4C). Then, when the stimulating electrode was advanced rostroventrally (site "D"), the downward-rotatory type was found (Fig. 4D). In the most rostroventral molecular layer (site "E"), the upward type (upward movement of both eyes) was discovered (Fig. 4E). In the most ventrorostral site in addition to the most rostral track (site "F") the combined upward and horizontal type (upward movement of both eyes combined with adduction of the right eye and abduction of the left eye) was found.

Thus, the downward-rotatory type was found in most parts of the rostral zone, the rotatory type was found in the border area between the rostral zone and the cerebellar peduncle, and the upward and combined upward and horizontal types were found in the border area between the rostral zone and the eighth nerve. Similar findings were obtained in 5 other cats in which the rostral zone was extensively explored (not illustrated).

Current spread

It was reported that stimulation of the vestibular nerve evoked contralateral horizontal eye movement.[2] In order to elicit this movement, the stimulation electrode was advanced through the rostral zone of the flocculus to the vestibular nerve lying just beneath the flocculus. Current spread was roughly estimated from depth-threshold curves for the contralateral horizontal component of the evoked eye movement (Fig. 5). The slopes of these curves investigated on 5 tracks were 80.0–120.0 μA per 1 mm depth. Therefore, we estimated that a current of 50 μA would spread 0.42–0.63 mm in the cerebellum. As mentioned above, the threshold currents for evoking eye movement were 5-20 μA when the stimulation sites were located in the Purkinje cell layer. A cur-

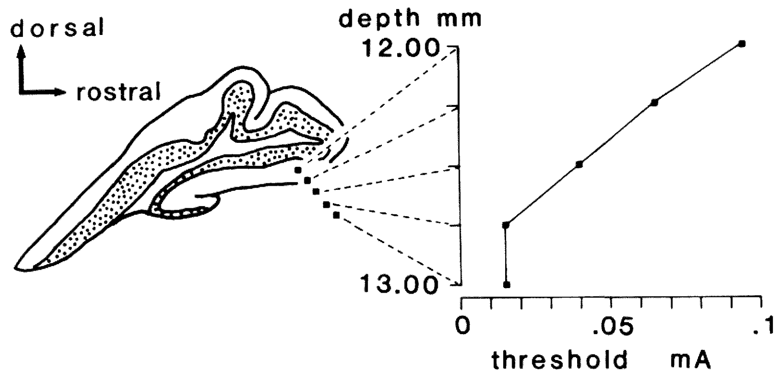

Fig. 5. Depth-threshold curves for the contralateral horizontal component of the eye movement evoked by stimulation of the sites indicated by the filled squares at depths of 12.00, 12.25, 12.50, 12.75, and 13.30 mm from the surface of the cerebellum.

rent of 20 μA applied to the Purkinje cell layer would not spread to the neighboring sites 0.25 mm away.

DISCUSSION

One of the anatomical principles of the zonal organization of the cerebellum is that the zones are perpendicular to the long axes of the folia.[1] In most parts of the cerebellum, the long axes of the folia are mediolaterally oriented, and the zones are rostrocaudally oriented (sagittal zones).[4] On the other hand, the folia of the cat flocculus are rather crooked. They are rostrocaudally oriented in the relatively caudal part of the flocculus and mediolaterally oriented in the relatively rostral part of the flocculus, resulting in crooked zones which are mediolaterally oriented in the relatively caudal part of the flocculus and rostrocaudally oriented in the relatively rostral part.[15] Therefore, if one tries to fit the present eye movement map into the rostrocaudally oriented sagittal zones, one would be confused by the complexity of the eye movement map. The eye movement map represented in the present study fits into the crooked zones of the cat flocculus reported in our previous study.[1]

The present investigation demonstrated that: (1) stimulation of the middle zone on one side evoked abduction of the ipsilateral eye and adduction of the contralateral eye; and (2) stimulation of the rostral or caudal zones of the flocculus on one side evoked a downward movement of both eyes combined with intorsion of the contralateral eye and a very small amplitude of the extorsion of the ipsilateral eye. These conclusions are summarized in Fig. 6. In addition, stimulation of the border areas between the

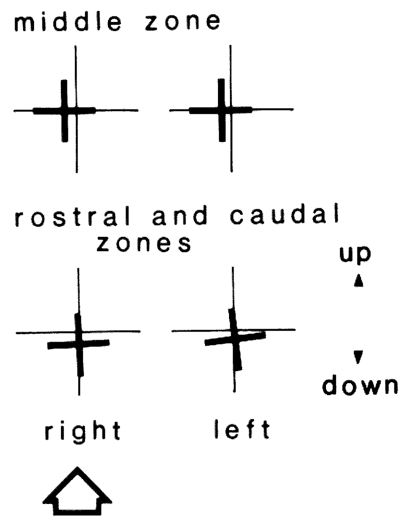

Fig. 6. Summarizing diagram showing the horizontal (upper) and downward- rotatory (lower) types of movement of both eyes evoked by stimulation of the rostral, middle and zonal zones of the flocculus. Deviation of crosses attached to corneas during stimulation (thick lines) from the primary position before stimulation (thin lines) is shown. An arrow represents the site of floccular stimulation.

rostral zone and the cerebellar peduncle evoked extorsion of the ipsilateral eye and intorsion of the contralateral eye, and between the rostarl zone and the eighth nerve evoked the upward movement of both eyes or a combined upward movement of both eyes, adduction of the ipsilateral eye and abduction of the contralateral eye. We could not exclude the possibility that the eye movement evoked by stimulation of these border areas was caused by current spread from the flocculus to the cerebellar peduncle or the eighth nerve.

In our previous investigation,[3] we reported that electrical stimulation of the caudal, middle, and rostral zones of the cat flocculus elicited downward, ipsilateral horizontal, and upward eye movements, respectively. Since movement of unilateral eye was recorded by electrooculography, neither rotatory component nor conjugacy of the evoked eye movement were fully investigated.[3] The present experiment complemented our previous experiment and provided new data concerning the rotatory component and conjugacy of the evoked eye movement. In addition, the results obtained in the present experiment, which extensively explored the rostral zone by the electrode, showed that stimulation of most parts of the rostral zone evoked a downward (not upward) eye movement. Due to technical restraints, no quantitative analysis of the eye movement in terms of velocities, dynamics, or trajectories is provided in the present experiment. However, our previous study[13] supplies the lacking quantitative data. The floccular stimulation by a train of pulses (intratrain frequency, 200 Hz; intensity, 30 μA; and duration, 1 sec) evoked slow and smooth eye movements of 2-3 deg/sec, the latency being 30-100 ms. With this stimulus, nystagmus was scarcely observed. In the present study, the most caudal part of the caudal zone was not explored with the stimulating electrode. However, our previous study,[3] which extensively explored the caudal zone by the electrode, showed that stimulation in the most caudal part of the caudal zone evoked a downward eye movement.

Neuronal pathways from the caudal,[5] middle,[6] and rostral (Sato and Kawasaki, in press) zones of the cat flocculus to the extraocular muscles have already been demonstrated. From these neuronal pathways and the action of the muscle, (i.e. the action of the lateral rectus muscle is abduction of the eye ball), it was expected that the increased activity of Purkinje cells in the rostral or caudal zones on one side resulted in a downward movement with an accompanying smaller amplitude of extorsion of the ipsilateral eye and intorsion with an accompanying smaller amplitude of downward movement of the contralateral eye (Sato and Kawasaki, in press). It was also expected that increased Purkinje cell activity in the middle zone on one side resulted in abduction of the ipsilateral eye and adduction of the contralateral eye (Sato and Kawasaki, in press). The present results showed that: (1) the expected downward movement of both eyes, intorsion of the contralateral eye, and extorsion of the ipsilateral eye were evoked by unilateral stimulation of the rostral or caudal zones; and (2) the expected abduction of the ipsilateral eye and adduction of the contralateral eye were evoked by unilateral stimulation of the middle zone.

Ron and Robinson[7] reported that stimulation of the monkey flocculus evoked ipsilateral horizontal, upward, and downward slow eye movement followed by nystagmus. Balaban and Watanabe[8] further suggested the existence of longitudinal zones in the monkey flocculus in relation to the direction of the evoked eye movement. An eye movement map was demonstrated also in the rabbit flocculus.[9,10] This map resembles ours in that the "H" zone from which stimulation evoked conjugate ipsilateral horizontal eye movements was flanked by two "V" zones from which stimulation evoked conjugate vertical eye movements. Species differences between the rabbit and cat show that: (1) in the rabbit, there are two separate zones ("R" and "V" zones) from which stimulation evoked conjugate rotatory and downward eye movements, respectively, while (2) in the cat, rotatory and vertical components were simultaneously evoked and never separated from each other.

Belknap and Noda[3] stimulated the monkey flocculus at low current (less than 22 μA) and observed evoked small eye movement of less than 2 deg. They could not find longitudinal zones in relation to the direction of the evoked eye movement, and proposed the existence of homogeneous subregions of the floccular cortex in contrast with the findings of other studies[8-10] including the present study. Because the thresholds of the evoked eye movement were low in the granular layer and high in the Purkinje cell layer, stimulation may have activated afferent fibers in the flocculus, while antidromic impulses excited relay neurons to the extraocular motoneurons through axon collaterals in their study. It has been reported that there are no prominent differences in mossy fiber afferents in floccular zones.[12] In addition, the thresholds for evoking eye movement in the present experiment were low in the Purkinje cell layer and high in the granular layer.

Graf, Simpson, and Leonard[13] have investigated the activity of floccular Purkinje cells in response to full-field visual rotation by a planetarium projector in the rabbit. The orientation of the best response axes of the rotation have a close geometrical relation with the best response axes of the anterior and horizontal semicircular canals of the ipsilateral labyrinth. On the basis of this geometric similarity, they have concluded that: (1) the flocculus has an intrinsic reference frame whose spatial orientation is strikingly similar to that of the semicircular canals of the vestibular system; and (2) the flocculus simply transforms sensory (retinal slip) input into motor (eye movement) output through the use of this intrinsic reference frame. The results of the present investigation support the conclusion by Graf et al., because the eye movement of the downward-rotatory and horizontal types evoked by unilateral stimulation of the flocculus on the righe side in the present experiments are the rotation on the plane of the anterior and horizontal canals on the right side, respectively.[5]

Voogd[4] reported that the cat cerebellar cortex can be divided into several sagittally oriented zones on the basis of differential efferent projections to the cerebellar and vestibular nuclei. These Purkinje cell zones receive differential climbing fiber afferents.[15,18] It is not known, however, whether the functional differences of these Purkinje cell/climbing fiber zones are present or not. The present investigation, in addition to our previous studies,[1-3,5,6,12,19] strongly suggests that functional differences exist in Purkinje cell/climbing fiber zones of the cat flocculus. Anatomically defined rostral and caudal zones may be related to eye movement control on the vertical plane. The middle zone may be responsible for eye movement control on the horizontal plane.

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