J. Physiol. (1977), 265, *pp.* 833–854
With 7 text-figures
Printed in Great Britain

SPECIFIC PATTERNS OF NEURONAL CONNEXIONS INVOLVED IN THE CONTROL OF THE RABBIT'S VESTIBULO-OCULAR REFLEXES BY THE CEREBELLAR FLOCCULUS

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(*Received 12 August 1976*)

SUMMARY

1. In anaesthetized albino rabbits, the occurrence of Purkinje cell inhibition on canal-ocular reflexes was surveyed with a reflex testing method.

2. Test reflexes were elicited by electrical stimulation of the semicircular canals. The results were appraised by recording potentials and tension from extraocular muscles. Twelve reflexes were defined in terms of the receptor canal and the effector muscle.

3. Conditioning electrical stimuli were applied to the flocculus, the inferior olive, and optic pathways at the retinae, optic chiasm, pretectal area and upper medulla.

4. The conditioning stimulation at the ipsilateral flocculus induced depression in six of the twelve canal-ocular reflexes; four of the six arose from the anterior canal and the remaining two from the horizontal canal.

5. The effect of stimulation of the contralateral inferior olive was similar to that of the ipsilateral flocculus, though less clear in two of the four reflexes from the anterior canal because of a contaminating effect.

6. The two reflexes from the horizontal canal were depressed by stimulation of the ipsilateral optic pathway which reached the ipsilateral flocculus via the contralateral pretectal area and inferior olive.

7. The four reflexes from the anterior canal were affected by stimulation of optic pathways in a different manner from each other. One was depressed from the contralateral retina via the ipsilateral pretectal area, while another was depressed from the ipsilateral retina via the contralateral pretectal area, though only occasionally. The third reflex was depressed from the ipsilateral pretectal area but not from the retina. The fourth was affected from neither the retina nor the pretectal area.

8. On the basis of latency measurements, it was concluded that the depression of canal-ocular reflexes was due to inhibition of relay neurones of the testing reflexes by flocculus Purkinje cells which were activated either directly, or indirectly through olivocerebellar climbing fibre afferents.

9. The above conclusion was supported by the observation that the depression induced by stimulation of the inferior olive and optic pathways was abolished by acute destruction of the ipsilateral flocculus.

10. The possible functional significance of the specific patterns of connexions from flocculus Purkinje cells to canal-ocular reflex pathways is discussed, and specialization among flocculus Purkinje cells in relationship with vestibulo-ocular reflexes is postulated.

INTRODUCTION

Just as in other reflex systems, the vestibulo-ocular reflex arc is formed of highly specific connexions from receptors (semicircular canals and otolith organs) to effectors (extra-ocular muscles) through relay neurones and motoneurones. Signals elicited from each canal thus evoke contraction in two extraocular muscles, one in each eye, and also relaxation in two muscles antagonistic to the contracting ones (Szentágothai, 1950; Cohen, Suzuki & Bender, 1964; Ito, Nisimaru & Yamamoto, 1976*a*, *b*, *c*). It is also well known that Purkinje cells in the cerebellar flocculus project to the vestibular nuclei (Dow, 1938; Angaut & Brodal, 1967) and inhibit relay neurones of the vestibulo-ocular reflex arc (Ito, Highstein & Fukuda, 1970; Fukuda, Highstein & Ito, 1972; Baker, Precht & Llinás, 1972; Highstein, 1973). Since not all of the relay neurones are inhibited (Fukuda *et al.* 1972), it may be that the flocculus inhibition is related specifically to certain labyrinthine receptors and/or extraocular muscles.

Purkinje cells in the flocculus are driven very effectively by signals elicited in the retina and mediated through climbing fibre afferents that originate from the inferior olive (Maekawa & Simpson, 1973). The major area of the flocculus is activated in this way from the ipsilateral retina, but the dorsorostral portion of the flocculus receives signals arising from the contralateral retina (Maekawa & Takeda, 1976). It may be that these two retinal projections to the flocculus are also related specifically to different labyrinthine receptors and/or extraocular muscles.

In the present investigation, these possibilities were examined by means of the reflex testing method previously developed for the rabbit's vestibulo-ocular reflexes (Ito *et al.* 1976*a*, *b*, *c*). A part of the present results has been published in brief (Ito, Nisimaru & Yamamoto, 1973; Ito, 1975).

METHODS

Forty adult albino rabbits (3·0–4·5 kg body weight) were used. They were anaesthetized with α-chloralose (60 mg/kg) plus urethane (400–800 mg/kg) administered I.V. or in a few cases I.P. The animal was respired artificially through cannulation of

the trachea and warmed by an electric heating pad. Fixation of the animal's head and dissection of the inner ear for canal stimulation and of extraocular muscles for recording were the same as described previously (Highstein, Ito & Tsuchiya, 1971; Ito *et al.* 1976*a*, *b*). In order to stimulate the retina, the eye ball was incised across the cornea, and the lens and a part of vitreous body were removed. The iris was then stitched to a metal ring (*ca.* 5 mm in diameter) mounted on the animal frame. Eye balls were removed when there was no need to stimulate the retina. Partial craniotomy was performed at four places: (1) over the paraflocculus of the cerebellum; (2) over the vermis of the cerebellar posterior lobe; (3) over the cerebral occipital lobe and (4) above the optic chiasm. The dorsal surface of the lower medulla was exposed by opening the atlanto-occipital membrane. In some experiments, the flocculus was extirpated by suction through a glass pipette (*ca.* 0·5 mm diameter) inserted posteriorly through the paraflocculus. The cerebral occipital lobe was removed by suction when the superior colliculus and neighbouring structures were to be exposed.

Bipolar electrodes made of enamelled stainless wire (40 μm diameter: Cohen *et al.* 1964) were used for stimulation of the semicircular canals. For bipolar stimulation of the whole labyrinth, two metal needle electrodes were inserted into the oval window of the cochlea. Duration and intensity of the stimulation of the canal or the whole labyrinth were adjusted by observing reflexes evoked in extraocular muscles (Ito *et al.* 1976*a*, *b*). The flocculus was approached posteriorly through the paraflocculus and stimulated bipolarly with two needles glued together, with tip separation of 0·5–1·0 mm or sometimes monopolarly with a single needle. In order to stimulate the inferior olive, a metal needle electrode was inserted into the medulla at 0·5 mm lateral to the mid line and 1–3 mm rostral to the obex. For monopolar stimulation of optic pathways, a needle was placed on exposed surfaces of the superior colliculus and neighbouring brain structures (Fig. 5*D*). The optic chiasm was stimulated bipolarly with a pair of needles separated by 1–2 mm and inserted dorsoventrally. The retina was stimulated bipolarly with a similar pair of needles inserted through the pupil. In cases of monopolar stimulation, the anode was a silver wire placed on the neck or temporal muscles. Currents passing through needle electrodes were monitored by recording the voltage drop across a 1 kΩ resistor inserted in series with anode.

Potentials were recorded from extraocular muscles with metal needle electrodes about 10 mm long and laquered except for about 1 mm at the tip (Ito *et al.* 1976*a*, *b*). Two needles were inserted into each muscle, one near the distal free end and the other at the muscle belly. These electrodes floated with the muscle and were connected to an amplifier through thin enamelled wire. Muscle tension was recorded with a mechano-electrical transducer (5737/M3514 Toshiba). When the retina or optic chiasm was stimulated, a ball-tipped silver wire electrode was placed on the occipital cerebral lobe to monitor evoked potentials in the visual cortex. Recorded potentials were displayed on the screen of a storage oscilloscope (TEKTRONIX 520). A data processing computer (ATAC, Nihonkoden 501–10) was used to average recorded potentials.

Reflex testing

Excitatory vestibulo-ocular reflexes were evoked by applying current pulses of relatively long duration (3–5 msec) to the labyrinth. The result of this stimulation was the appearance of synchronized discharges in certain extraocular muscles (Ito *et al.* 1976*a*). Inhibitory vestibulo-ocular reflexes were similarly evoked, but, since the duration of stimulating pulses for this effect had no threshold (Ito *et al.* 1976*b*), brief pulses of 0·1–0·5 msec duration were preferred. The end-effect of the inhibitory vestibulo-ocular reflexes was appraised by appearance of the *slow muscle potentials*

and of concomitant transient decrease of the tension in certain extraocular muscles (Ito *et al.* 1976*b*). The *slow muscle potentials* are indicative of suppression of spontaneous nervous discharges into extraocular muscles due to post-synaptic inhibition of oculomotor neurones (Ito *et al.* 1976*b*, *c*). Twelve principal components of the vestibulo-ocular reflexes, six excitatory and six inhibitory, were defined in this way in terms of the receptor canal–effector muscle interaction, as listed in Table 1.

These excitatory and inhibitory vestibulo-ocular reflexes were used as test responses to detect any inhibition induced in relay neurones of these reflexes through Purkinje cells of the flocculus. To provide sensitive testing, amplitudes of the testing responses usually were kept submaximal, about 10 % of the maximum. For the initial survey, the reflexes were evoked by stimulation of the whole labyrinth, and for later confirmation of positive results derived from the initial survey they were evoked individually by selective stimulation of single canals. Laterality always refers to the side of the canal stimulation.

Abbreviations

i–, ipsilateral to the canal stimulated for testing; c–, contralateral to the canal stimulated for testing; SR, superior rectus; SO, superior oblique; MR, medial rectus; IR, inferior rectus; IO, inferior oblique; LR, lateral rectus.

RESULTS

Direct flocculus stimulation

As a stimulating electrode was inserted through the paraflocculus on the side of testing canals, the following effects emerged regularly at the depths of the flocculus (7–10 mm from the caudal surface of the paraflocculus). The threshold for inducing these effects was usually as low as 0·1–0·2 mA for brief pulses of 0·2 msec duration. In a few experiments, location of the stimulating electrode within the white matter of the flocculus was verified by histological examination (cf. Fukuda *et al.* 1972).

Excitatory vestibulo-occular reflexes

In Fig. 1*A*, the testing reflex from the horizontal canal to the i-MR muscle was evoked by stimulation of the labyrinth and was depressed very effectively by conditioning stimulation at the ipsilateral flocculus. The time course of the depression was plotted in Fig. 1*C* as a function of the conditioning-testing time intervals measured from the moment of onset of the flocculus stimulation to the foot of test reflex discharges (see Fig. $1A_4$). The depression started shortly following the flocculus stimulation and lasted for about 4 msec. The duration of the depression varied from case to case up to 30 msec. By contrast, no depression was detected in the reflex from the posterior canal to the i-SO muscle simultaneously tested (Fig. 1*B* and *C*). Stimulation of the ipsilateral flocculus also very effectively depressed the reflex from the anterior canal to the i-SR muscle (Fig. 1*D*) and that to the c-IO muscle (not illustrated), but other excitatory reflexes were not affected. These results are summarized in Table 1.

TABLE 1. Occurrence of depression in canal-ocular reflexes following stimulation of the flocculus and related structures. +, presence of the depression. −, absence of the depression. *, inconclusive because of complicating potential changes evoked by conditioning stimuli. Figures attached to these symbols indicate the number of preparations used. Triangles represent that acute flocculotomy abolished the depression. The number of triangles means the number of preparations tested. i-FL, ipsilateral flocculus. c-OL, contralateral inferior olive. c-PA, contralateral pretectal area. i-PA, ipsilateral pretectal area. OX, optic chiasm. i-RE, ipsilateral retina. c-RE, contralateral retina. C., Canal

Test reflexes				Depression following stimulation of						
Action	No	Receptor	Muscle	i-FL	c-OL	i-RE	c-RE	OX	c-PA	i-PA
Excitatory	E_1	Anterior c.	i-SR	+8	+8▲	−5	−7	−7	−5	+4 −7▲
	E_2		c-IO	+6	+7▲	−6	+5 −4▲	+3 −4	−5	+10 −2
	E_3	Horizontal c.	i-MR	+10	+9▲▲	+10▲	−5	+9	+12▲	−5
	E_4		c-LR	−3	−3		−1	−4	−3	−3
	E_5	Posterior c.	i-SO	−3	−4			−4	−3	−3
	E_6		c-IR	−4	−4			−3	−3	−4
Inhibitory	I_1	Anterior c.	i-IR	+7	*4	*3 −2	−5	*3 −3	*1 −5	−5
	I_2		c-SO	+4	*3	+1 −5	−5	+1 −4	+1 −5	*3 −2
	I_3	Horizontal c.	i-LR	+3	+3▲	+5▲	−5	+4	+5▲	−5
	I_4		c-MR	−3	*3	−2		−1	−1	−1
	I_5	Posterior c.	i-IO	−3	*1 −2			−2	−2	−1
	I_6		c-SR	−3	*1 −2			−2	−2	−1

It is noted there that negative results were confirmed in more than three preparations for each muscle. In a few experiments, contralateral flocculus stimulation was also employed for conditioning, but no appreciable effect was elicited in any canal-ocular reflex.

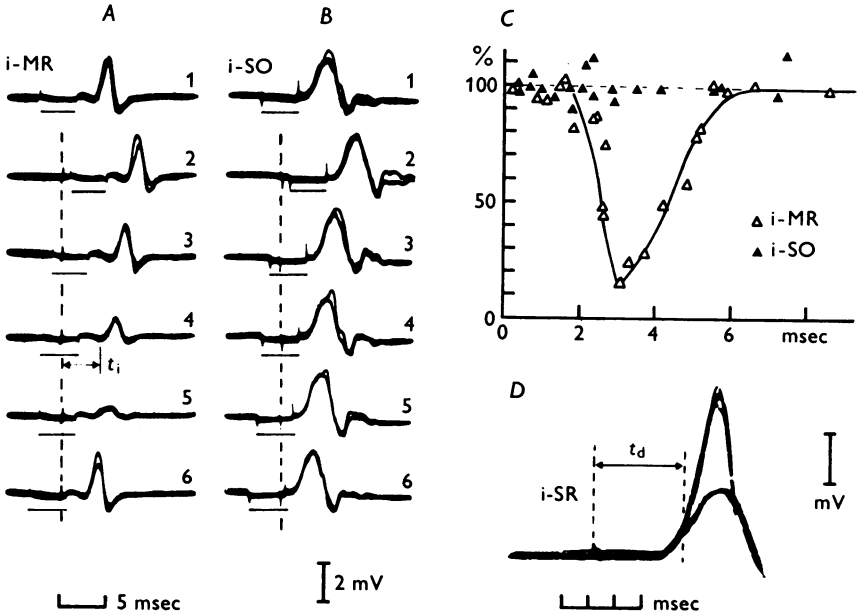

Fig. 1. Depression of excitatory canal-ocular reflexes caused by direct stimulation of the flocculus. *A* and *B*, reflex discharges recorded from two muscles indicated. Horizontal bar attached below each record indicates the period of application of stimulating currents to the labyrinth. Vertical interrupted lines mark the moment of stimulation at the flocculus ipsilateral to testing canals. A_1 and B_1, control testing responses. From A_2 to A_6, and from B_2 to B_6, timing of the testing stimulation relative to the flocculus stimulation was gradually shifted. The method of measuring the conditioning-testing intervals (t_i) is indicated in A_4 (see text). *C* plots peak amplitudes of reflex discharges relative to their control sizes as function of conditioning-testing intervals, on the series partly illustrated in *A* and *B*. *D*, reflex discharges in the muscle indicated. Responses with and without flocculus stimulation were superimposed. Vertical interrupted line to the left marks the moment of the flocculus stimulation. That to the right is on the diverging point between the two response curves. t_d, latency of the depression according to the method of Araki *et al.* (1960). In Fig. 1*A* and *B* as well as in Figs. 2*A* and *B*, and 3*A*, records were taken by superimposing 5–10 sweeps repeated at a rate of 1/sec. In recording potentials from muscles, upward deflexion always represents negativity in the proximal electrode relative to the distal one (Methods). Voltage scale in *B* is common to *A* and *B*. Time scale in *A* also applies to *B*.

Inhibitory vestibulo-ocular reflexes

In Fig. 2*A* and *B*, the *slow muscle potentials* and the transient decrease of muscle tension were simultaneously recorded and indicated the inhibitory vestibulo-ocular reflex in the c-SO muscle evoked by stimulation of the anterior canal. Marked depression occurred in this inhibitory reflex shortly following flocculus stimulation and lasted for more than 20 msec, as shown in Fig. 2*A* and *B* and plotted in *C*. In Fig. 2*C*, amplitudes of the

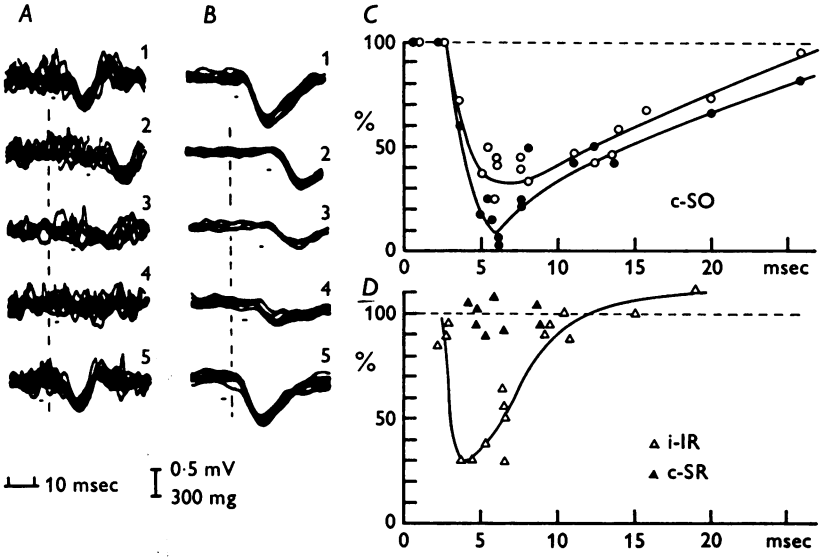

Fig. 2. Depression of inhibitory canal-ocular reflexes caused by direct stimulation of the flocculus. *A*, *slow muscle potentials* and *B*, transient decrease of the muscle tension simultaneously recorded from c-SO muscle. Horizontal bars underneath records indicate similarly to Fig. 1*A* and *B*. A_1 and B_1, control responses. From A_2 to A_5 and from B_2 to B_5, the moments of testing stimulation were shifted gradually relative to that of the flocculus stimulation (vertical interrupted line). Voltage scale is 0·5 mV for *A* and 300 mg for *B*. Time scale of *A* applies also to *B*. *C* plots amplitudes of the *slow muscle potentials* (●) and transient tension decrease (○) relative to their control values against the conditioning-testing intervals, for the series partly shown in *A* and *B*. How to measure the conditioning-testing intervals for these inhibitory reflex responses is indicated in the text. *D* plots similarly but for the muscles indicated which were tested in the same preparation as for *A–C*.

testing *slow muscle potentials* were indicated as a function of the conditioning-testing time intervals measured from the moment of onset of the flocculus stimulation to the foot of the *slow muscle potentials*. Amplitudes of the testing transient tension decrease are plotted also against the time

intervals determined for the *slow muscle potentials* simultaneously tested. As expected, the time course of the depression in the *slow muscle potentials* was very similar to that in the transient tension decrease. Fig. 2*D* illustrates that similar depression occurred in the reflex from the anterior canal to i-IR muscle, while there was no sign of depression in the reflex from the posterior canal to the c-SR muscle examined in the same preparation. As summarized in Table 1, stimulation of the flocculus also depressed the inhibitory reflex from the horizontal canal to the i-LR muscle, but other inhibitory reflexes were not affected.

Stimulation of the inferior olive

As the stimulating electrode reached the depths of the inferior olive (3–4 mm from the dorsal surface of the medulla) on the side contralateral to the testing canals, marked depression was caused in certain vestibulo-ocular reflexes, as shown in Fig. 3*A* and *B* for the excitatory reflex in i-SR muscle. As seen in Table 1, similar depression occurred in all of the three excitatory reflexes affected by direct flocculus stimulation. The threshold for inducing the depression was often as low as 0·05–0·1 mA for brief pulses of 0·2 msec duration. The latency of the reflex depression induced by olivary stimulation was about 4 msec longer than that induced by direct flocculus stimulation (compare Fig. 3*B* with Fig. 1*C*; see below).

Before the onset of the depression after the olivary stimulation, a facilitation phase was sometimes seen to occur as shown in Fig. 3*B*. This facilitation may correspond to small excitatory post-synaptic potentials which precede the inhibitory post-synaptic potentials evoked in Deiters and cerebellar nuclei neurones by stimulation of the inferior olive (Ito, Obata & Ochi, 1966; Ito, Yoshida, Obata, Kawai & Udo, 1972) and which have been ascribed to synaptic connexion to these neurones formed through axon collaterals of olivocerebellar fibres. The rebound facilitation following the depression in Fig. 3*B* may also be related to the post-synaptic events observed in Deiters neurones; the post-synaptic inhibition induced by stimulation of the inferior olive was followed by a late depolarization. This depolarization was attributed to a decrease of the tonic discharges from Purkinje cells (Ito *et al.* 1966).

Stimulation of the inferior olive also depressed the inhibitory vestibulo-ocular reflex from the horizontal canal to the i-LR muscle, as illustrated in Fig. 3*C* and *D*. The latency of this depression was comparable with that described above for the excitatory reflexes. Testing with the *slow muscle potential* in other muscles, however, was difficult because the olivary stimulation by itself tended to produce *slow muscle potentials* of comparable size, as shown in Fig. 3*E–G*. This effect of olivary stimulation could be mediated by inhibitory relay neurones of the vestibulo-ocular reflexes or otherwise of other types of inhibitory neurones. These inhibitory neurones could be excited by impulses elicited in axon collaterals of olivocerebellar fibres or in other neuronal elements running nearby the

inferior olive. Hence, even when testing *slow muscle potentials* superposed on these potentials were reduced in size, it was not possible to judge whether it was due to genuine depression or merely to occlusion at inhibitory relay neurones, or to saturation of post-synaptic inhibition at oculomotor neurones. Those cases with inconclusive results are indicated by * in Table 1.

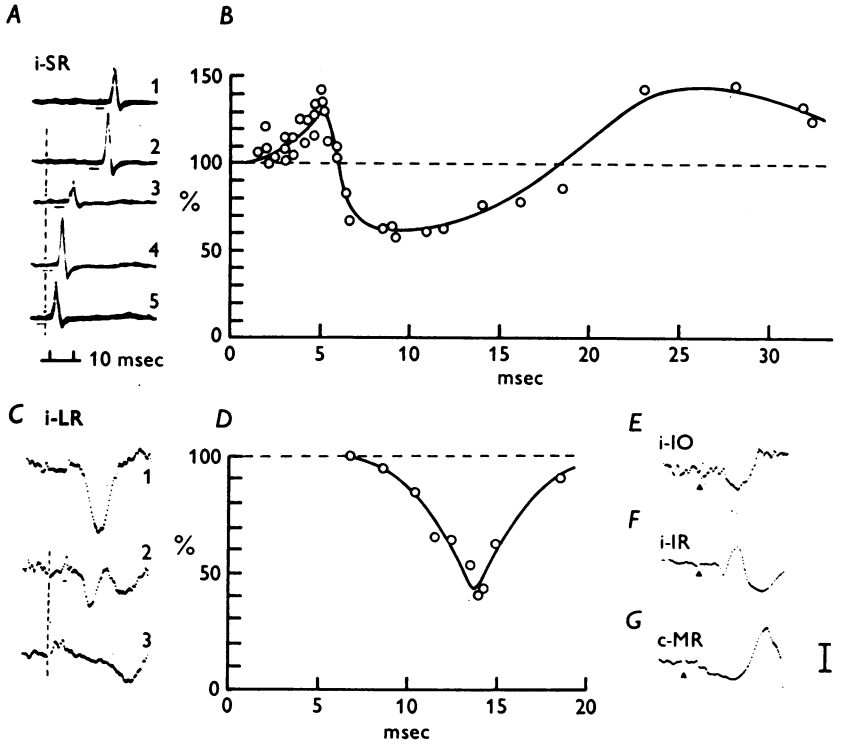

Fig. 3. Depression of canal-ocular reflexes induced by stimulation of the inferior olive. *A*, specimen records taken from the muscle indicated. Testing stimuli (horizontal bars) were applied to the anterior canal. Vertical interrupted line indicates the moment of the olivary stimulation. *B* plots reflex response-interval relationship for the series partly illustrated in *A*. *C*, *slow muscle potentials* recorded from the indicated muscle. C_1, control test response. C_2, testing after conditioning olivary stimulation at the moment indicated by vertical interrupted line. C_3, potential changes induced by the olivary stimulation alone. Records in *C* were obtained by averaging *slow muscle potential* during 20 successive sweeps repeated at a rate of 1/sec. *D*, response–interval curve for the series partly illustrated in *C*. *E–G*, potential changes induced in the indicated muscles by stimulation of the inferior olive alone. Potentials were averaged 20 times in *E* and *F*, and 50 times in *G*. Voltage scale in *G* is 0·5 mV for *A*, 0·1 mV for *C*, *E*, *F* and 0·2 mV for *G*. Time scale of 10 msec is common to all records of Fig. 3. In *E–G* the moment of onset of the olivary stimulation is indicated by triangles.

Stimulation of the retina and optic chiasm

When the ipsilateral retina was stimulated, marked depression was regularly induced in the excitatory reflex from the horizontal canal to the i-MR muscle, as illustrated in Fig. 4 *A*. With maximal stimulus intensity for evoked potentials recorded in the visual cortex, the depression occurred with single shock stimulation of the retina. However, in order to obtain stable and prominent depression, double shock stimulation with an interval of 2·0 msec was routinely used. The latency of onset of the reflex depression after the first of the two stimuli was virtually the same as that obtained with single shock stimulation. It was as long as 10–15 msec on the response–interval curves as exemplified in Fig. 4 *A*.

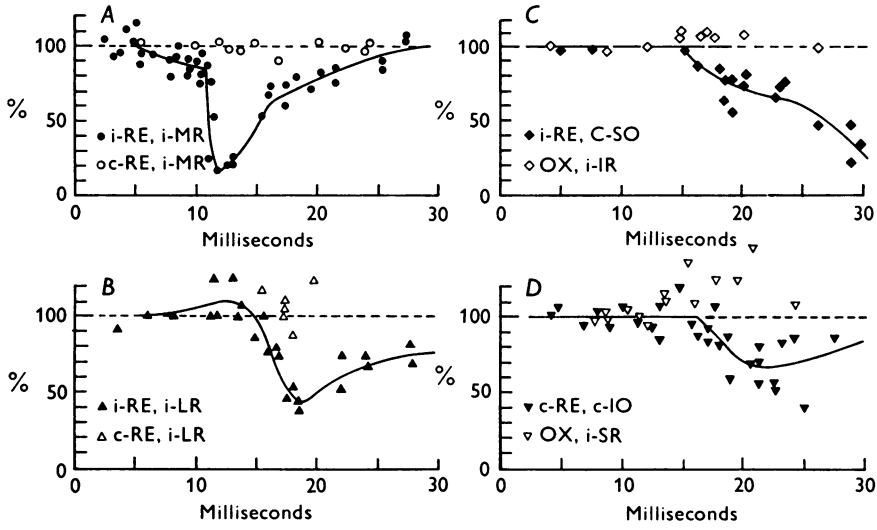

Fig. 4. Depression of canal-ocular reflexes following stimulation of the retina. Similar plotting to Figs. 1*C* and 2*C* and *D*. The retina stimulated and the muscle from which reflexes were recorded are indicated for each symbol. i-RE, c-RE and OX are defined in the legend of Table 1.

Prominent depression with a comparable latency was induced regularly also in the inhibitory reflex from the horizontal canal to the i-LR muscle, following stimulation of the ipsilateral retina, as shown in Fig. 4 *B*. Similar depression occurred in the inhibitory reflex to c-SO muscle, as shown in Fig. 4 *C*, though this effect was seen only in one out of the six preparations tested, as indicated in Table 1. It is also noted in Table 1 that observation in the inhibitory reflex to i-IR muscle was frequently disturbed by potential changes induced by the ipsilateral retinal stimulation. In two preparations, however, the complication was negligible, and it was confirmed that

there was no depression in this reflex following the ipsilateral retinal stimulation.

Stimulation of the contralateral retina produced no appreciable depression in those reflexes from the horizontal canal to the i-MR and i-LR muscles (Fig. 4 *A* and *B*), but it did depress the excitatory reflex from the anterior canal to the c-IO muscle, as shown in Fig. 4 *D*. This contralateral effect was rather unstable in the sense that it was absent in four of the nine preparations tested (Table 1). Stimulation of the contralateral retina did not produce depression in the other excitatory reflex from the anterior canal to the i-SR muscle, as indicated in Fig. 4 *D*. None of the three inhibitory reflexes depressed by direct flocculus stimulation was affected by the contralateral retinal stimulation (Table 1).

Effects of stimulation of the optic chiasm were merely the sum of those produced from the ipsilateral and contralateral retina, as shown in Table 1. Depression occurred in the excitatory reflexes to the c-IO and i-MR muscles and in the inhibitory ones to i-LR muscle. It occurred also in one case in the inhibitory reflex to c-SO muscle, but never in other reflexes.

Stimulation of the pretectal area and the upper medulla

Retinal signals to the flocculus are mediated by the pretectal areas (Maekawa & Simpson, 1973; Maekawa & Takeda, 1976). When the surface of the contralateral pretectal area was stimulated with a monopolar electrode, marked depression regularly occurred in the excitatory reflex to the i-MR muscle, as shown in Fig. 5 *A*. Two curves with a shorter (6 msec on the response–interval curve) and a longer latency (10 msec) are indicated in Fig. 5 *A*. The depression with the shorter latency could be induced with low threshold from a localized region of the pretectal area, as shown in Fig. 5 *C* and *D* (○). The depression with the longer latency arose from neighbouring spots with low threshold, as also indicated in Fig. 5 *C* (●). At the latter spots, the latency was abruptly shortened as the stimulus intensity was increased, as in Fig. 5 *A*. Apparently, the depression with the shorter latency was due to direct excitation of pretectal neurones, while that with the longer latency was caused by excitation of presynaptic fibres to these neurones. Stimulation of the contralateral pretectal area also regularly depressed the inhibitory reflex to the i-LR muscle, as shown in Fig. 5 *B*. Similar depression of the inhibitory reflex to the c-SO muscle was observed in the one preparation where the reflex was depressed by stimulation of the ipsilateral retina and optic chiasm (Table 1), but not in other preparations.

Stimulation at the ipsilateral pretectal area was effective in producing depression of the excitatory reflexes from the anterior canal to the c-IO and i-SR muscles, as shown in Fig. 6 *A*. The effect upon the c-IO muscle

was missing in two of the twelve preparations examined in which anaesthesia was relatively deep as judged by the absence of flexion reflexes in response to pinching of the skin. The effect upon the i-SR muscle was more labile, as it was present only in four of the eleven preparations tested (Table 1). Further, in two of the four preparations, the effect upon the

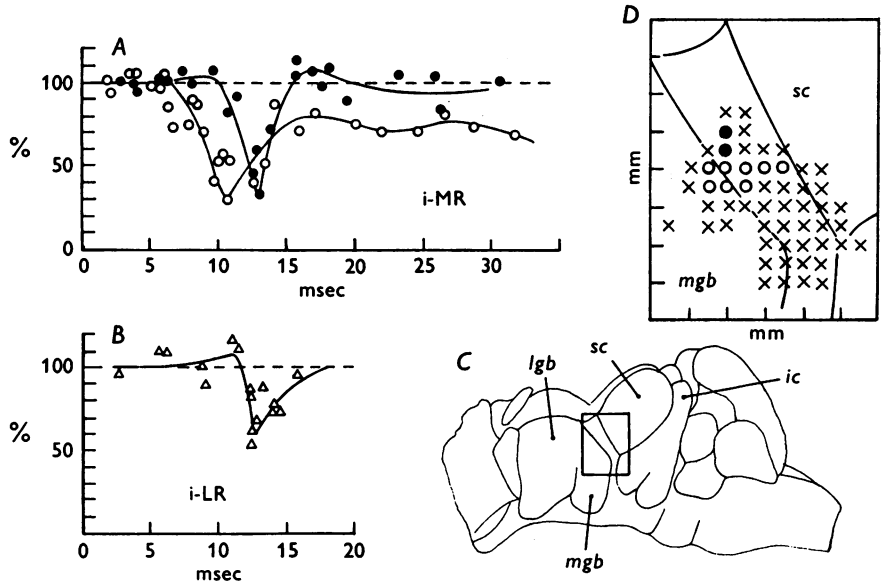

Fig. 5. Effects of stimulation at the contralateral protectal area. *A* plots the response-interval curve for the muscle indicated. Filled circles, monopolar stimulation with 100 μA (tip negative). Open circles, stimulation at the same site for filled circles, but with 500 μA. *B*, response–interval curve for the *slow muscle potential* in the muscle indicated. *C* illustrates the left side view of the brainstem. *sc*, superior colliculus; *ic*, inferior colliculus; *lgb*, lateral geniculate body; *mgb*, medial geniculate body. The pretectal area is enclosed by a rectangle and is enlarged in *D*. In *D* open circles indicate the spots from which the depression of the testing reflex evoked from the right horizontal canal in the i-MR muscle was elicited with a low threshold stimulus (40–100 μA) and with a relatively short latency. Points shown by filled circles, the threshold was similarly low, but the latency was longer by about 4 msec than that at open circles. Crosses mark those spots with threshold value higher than 120 μA.

i-SR muscle disappeared during the course of repeated trials of stimulation. No other reflexes were affected at all by stimulation at the ipsilateral pretectal area. Fig. 6*B* and *C* illustrates that the spots with low threshold for depressing the reflexes to the c-IO and i-SR muscles were located over the pretectal area, relatively ventrally in comparison with those for the reflex to i-MR muscle (Fig. 5*C*). The latency of onset of the depression

varied among these low threshold spots by a few milliseconds. Those spots where the shortest latency was obtained (⊙ Fig. 6*B* and *C*) probably represented the location of relevant pretectal neurones.

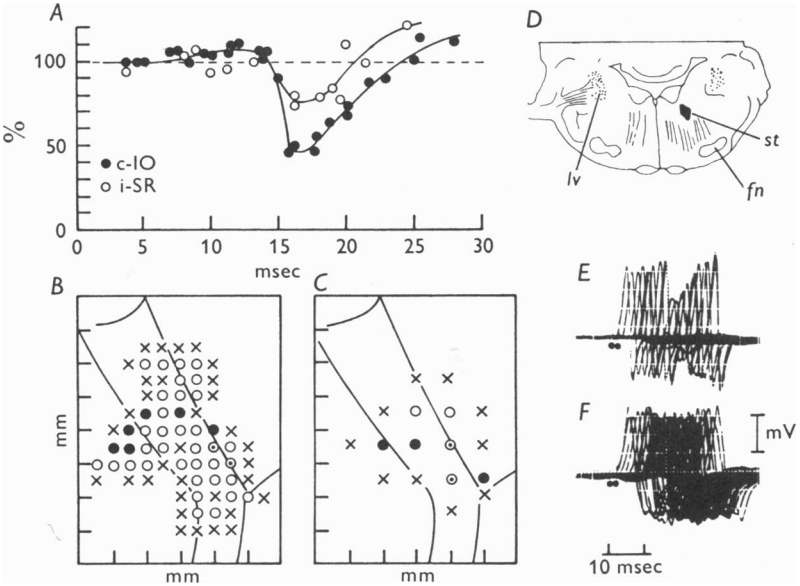

Fig. 6. Effects of stimulation at the ipsilateral pretectal area. *A* plots the response–interval curves for the two muscles tested simultaneously after conditioning stimulation at the surface of the ipsilateral area. *B* and *C* illustrate the left pretectal area as in Fig. 5*D*. Testing reflexes were evoked from the left anterior canal in the c-IO and i-SR muscles. In *B*, circles plot those spots from which the reflex to the c-IO muscle was depressed with threshold lower than 100 μA. Latency of the depression was shortest at ⊙ (14 msec as measured by the method of Araki *et al.*, see text) and longest at ● (16 msec), while it was intermediate (15 msec) at ○. *C* indicates similarly but for the reflex to the i-SR muscle. Note that Fig. 5*D* was mapped simultaneously with Fig. 6*B* by alternating the two reflexes during conditioning at each spot. Fig. 6*C* was obtained in a later trial on the same preparation. *D*, cross-section of the upper medulla. *lv*, Deiters nucleus; *fn*, nucleus n. facialis; *st*, electrolytic lesion made on the side contralateral to the testing canal, from which depression of the reflexes to the i-SR and c-IO muscles was induced with relatively low stimulus intensity of the monopolar stimulation (threshold below 100 μA) with a latency of about 10 msec in the response–interval curves. *E*, test reflexes evoked in c-IO muscle from the anterior canal and registered on the screen of a storage oscilloscope. The position of the test stimuli was gradually shifted, while the sweep was repeated once every second. Conditioning stimuli were applied to the ipsilateral pretectal area at the moments indicated by two dots. *F*, same as in *E*, but after acute destruction of the flocculus on the side of the test anterior canal.

TABLE 2. Latency values for the reflex depression caused by stimulation of the flocculus and related structures. Abbreviations are the same as in Table 1. Latency values were measured by the method described in Fig. 1 *D* (t_d). Calculated values were obtained in the following way. t_1, sum of the latency for flocculus Purkinje cells to inhibit relay neurones (1·1 msec as the middle value of the range 0·9–1·3 msec; Fukuda *et al.* 1972), that for impulse in relay neurones to produce post-synaptic effects in oculomotor neurones (1·0 msec; Highstein *et al.* 1971) and that for motoneuronal impulses to reach extraocular muscles (1·1 msec; Ito *et al.* 1976*c*). t_2–t_5, latencies for climbing fibre activation of flocculus Purkinje cells. t_2, 3·8 msec (middle value of 3·5–4·0 msec after olivary stimulation; Maekawa & Simpson, 1973). t_3, 8·1 msec (after stimulation underneath the surface of the contralateral pretectal area; Maekawa & Simpson, 1973). t_4, 12·0 msec (after stimulation of the ipsilateral pretectal area; K. Maekawa and T. Takeda, personal communication). t_5, 12·5 msec (middle value of 11–14 msec after stimulation of the ipsilateral retina; Maekawa & Simpson, 1973). t_6, 2·3 msec (middle of 2·0 and 2·5 msec, latency difference between the climbing fibre activation from the ipsilateral and that from the contralateral retina; Maekawa & Takeda, 1976)

Stimulated site	Muscle recorded from	Latency of the depression: Number of measurements		Range (msec)	Mean and S.D. (msec)	Calculated (msec)
i-FL	i-SR	3	9	3·0–3·4	3·4 ± 0·4	3·2 (t_1)
	c-IO	3		3·0–3·6		
	i-MR	3		3·0–4·2		
c-OL	i-SR	4	12	6·8–8·3	7·9 ± 0·4	7·0 (t_1+t_2)
	c-IO	5		7·6–8·0		
	i-MR	3		7·8–8·4		
c-PA	i-MR	9		9·5–14·3	11·9 ± 1·6	11·3 (t_1+t_3)
i-PA	i-SR	3	15	14·0–15·5	14·7 ± 1·1	15·2 (t_1+t_4)
	c-IO	12		13·0–16·9		
i-RE	i-MR	6		14·5–16·5	15·8 ± 0·8	15·7 (t_1+t_5)
c-RE	c-IO	5		16·8–19·0	17·9 ± 0·7	18·0 ($t_1+t_5+t_6$)

In the experiment of Fig. 6*D*, the upper medulla was systematically tracked with a monopolar stimulating electrode. The effective spot for depressing the reflexes from the anterior canal to the i-SR and c-IO muscles was localized to the contralateral side at about the position of the so-called central tegmental tract (cf. Maekawa & Simpson, 1973).

Latency of onset of the depression

The exact moment of onset of the depression induced in the excitatory reflexes was estimated by the method of Araki, Eccles & Ito (1960). In Fig. 1*D*, the flocculus stimulation was timed so that depression started on the rising phase of the testing reflex discharges. When allowance is made for the transmission time from relay neurones to extraocular muscles, the diverging point of the control and depressed reflex discharges indicates the moment of onset of flocculus inhibition at the relay neurones. Latency values thus obtained with stimulation of the flocculus, inferior olive, pretectal areas and retinae are shown in Table 2. For this measurement, the pretectal areas were stimulated with relatively strong stimuli (0·3–0·5 mA) in order to secure the direct excitation of pretectal neurones (see above).

The method of Araki *et al.* (1960) was not applied to the *slow muscle potentials* for the following reason. The post-synaptic inhibition in oculomotor neurones and the rate of spontaneous discharges from them are not linearly related with each other; as the former increases, the latter would be cut off to zero (see Fig. 3*F* of Ito *et al.* 1976*b*). This should introduce a significant discrepancy between the time course of the motoneuronal inhibition and that of the *slow muscle potentials*. Hence, it is not always certain that the diverging point between the control and depressed *slow muscle potentials* corresponds to that between the control and depressed motoneuronal inhibition.

Effect of the acute cerebellectomy

While the reflex depression was being demonstrated by stimulating the inferior olive or optic pathways, the paraflocculus and then the flocculus on the side of the testing canals were acutely extirpated by suction. Complete loss of the paraflocculus never affected the reflex depression, but as soon as the suction was extended into the flocculus, there was sudden and complete removal of the reflex depression as shown in Fig. 6*E* and *F* for the reflex to i-MR muscle. Relevance of the ipsilateral flocculus to the reflex depression was thus demonstrated for each of the combinations of the conditioning stimuli and testing reflexes where the depression was effectively induced (▲ in Table 1). The acute flocculotomy, however, was not performed for the depression of the inhibitory reflex to c-SO muscle,

because the depression of this reflex found in one experiment could not be reproduced in other experiments.

Acute flocculotomy on the side contralateral to the testing canals had no effect at all upon the depression caused from the inferior olive, as tested in four preparations. In one preparation, the effect of olivary stimulation was tested after extirpation of the anterior and posterior lobes of the cerebellum, with the flocculus and lateral parts of the cerebellar hemisphere left intact; depression remained unaffected.

DISCUSSION

That the depression of canal-ocular reflexes observed in the present investigation is due to the inhibitory action of flocculus Purkinje cells can be concluded from the latency values of the depression. Table 2 shows that the measured latencies were always close to those calculated from previous data as the time spent for impulse travel along pathways through the flocculus and inferior olive (see legend of Table 2). Even though the accurate latency measurement could not be performed on the inhibitory canal-ocular reflexes, time courses of their response–interval curves similar to those for excitatory reflexes (Figs. 2, 3, 4, 5) indicate that the inhibitory reflexes were also depressed by Purkinje cell inhibition. The fact that acute flocculotomy regularly abolished the depression of both excitatory and inhibitory canal-ocular reflexes evoked from the optic pathways strongly supported this view (Table 1).

With stimulation of the inferior olive, three lines of evidence were obtained to indicate that cerebellar areas other than the flocculus were not involved in the depression of canal-ocular reflexes presently studied: (1) olivary stimulation did not produce further depression than was evoked by stimulation of the flocculus (Table 1); (2) acute extirpation of the ipsilateral flocculus regularly abolished the whole effect of the olivary stimulation; (3) destruction of the anterior and posterior lobes of the cerebellum including the nodulus and uvula, but with the flocculus and lateral portions of the cerebellar hemisphere left intact, did not influence the effect of olivary stimulation.

Pattern of the specific projection from the flocculus

The present investigation disclosed that Purkinje cells of the cerebellar flocculus exert inhibitory action upon just half of the twelve major canal-ocular reflex pathways of rabbits, as summarized in Table 1. Partial support for the present results of Table 1 is available from the micro-electrode investigation performed on cat's trochlear (Baker *et al.* 1972; corresponding to E_5 and I_2 of Table 1) and abducens (Highstein, 1973; to E_4 and I_3)

motoneurones. Positive results with excitatory reflexes from the anterior canal (E_1 and E_2) are in good agreement with the observation that second-order vestibular impulses evoked in the IIIrd nucleus via the brachium conjunctivum were depressed by stimulation of the flocculus (Fukuda *et al.* 1972), as the anterior canal signals for these reflexes are conveyed through the brachium conjunctivum (Ito *et al.* 1976*a*). Positive results with the inhibitory reflexes from the anterior canal to the i-IR muscle (I_1) are also consistent with the previous finding that the inhibitory relay neurones projecting to the IIIrd nucleus were inhibited by stimulation of the flocculus (Fukuda *et al.* 1972). Occurrence of the flocculus inhibition upon the reflex from the horizontal canal to the i-MR muscle (E_3), however, was revealed for the first time by the present investigation, together with the absence of the flocculus inhibition in the three inhibitory reflexes to IIIrd nucleus motoneurones (I_4, I_5, I_6).

The flocculus inhibition is exerted on those reflexes arising from the anterior and horizontal canals but not on those from the posterior canal (Table 1). This corresponds very well to the histological observation that the monkey's flocculus receives primary vestibular afferents from the anterior and horizontal canals (and also from otolith organs) but not from the posterior canal (Carpenter, Stein & Peter, 1969). Since, however, the flocculus inhibition is exerted only upon two of the four reflexes arising from the horizontal canal, the primary receptor is not the only factor which determines the specific connexion from the flocculus to the canal-ocular reflex pathways. That the effector muscle is another factor is indicated in Fig. 7 where canal-ocular reflex pathways and inhibitory projection from the flocculus are illustrated in relationship to each extra-ocular muscle. Even though the laterality of the flocculus inhibition relative to the effector and the synaptic action of the inhibited relay neurones vary from muscle to muscle, there is one general rule; the flocculus acts upon only one, but never both, of the two major reflex pathways converging on to each muscle. It appears that the status of each muscle is held in a balance of antagonism between two major reflexes and that the flocculus controls this balance by influencing one of the two reflexes.

Optic pathways through the inferior olive and the flocculus

Maekawa & Simpson (1973) showed that signals from the ipsilateral eye reach the ipsilateral flocculus via the contralateral pretectal area and inferior olive. The present investigation established that this optic pathway from the ipsilateral eye to the flocculus is closely related to the horizontal canal-ocular reflexes to that eye (i-MR and i-LR, Fig. 7*E*, *F*). Maekawa & Takeda (1976) also demonstrated that signals from the contralateral retina pass to the ipsilateral pretectal area and that, after returning back

again to the contralateral side, they descend to the contralateral inferior olive, eventually reaching the ipsilateral flocculus. In the present experiment, this contralateral optic pathway to the flocculus was related to the reflex to the c-IO muscle (Fig. 7*D*).

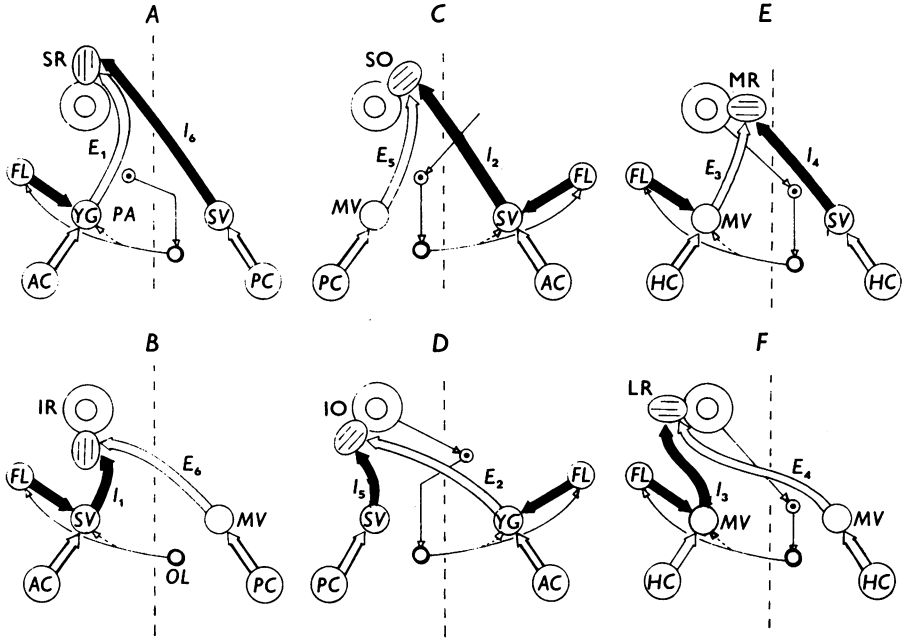

Fig. 7. Specific connexions for canal-ocular reflexes and their cerebellar control. The results summarized in Table 1 are rearranged to visualize their relevance to each muscle. *AC*, anterior canal; *PC*, posterior canal; *HC*, horizontal canal; *YG*, *y* group region of the vestibular nuclear complex (cf. Ito *et al.* 1976*a*); *MV*, medial vestibular nucleus; *SV*, superior vestibular nucleus. Hollow arrows represent excitatory action and those filled in black inhibitory one. *FL*, flocculus; *OL*, inferior olive; *PA*, pretectal area. In each diagram, vertical broken line indicates the mid line. Double circles represent an eye and an ellipsoid attached to each eye an extraocular muscle. Oculomotor neurones are omitted for simplification, and synaptic action from relay neurones on to oculomotor neurones are indicated by arrows drawn from relay neurones to extraocular muscles. The reflex numbers (E_1–E_6, I_1–I_6, in Table 1) are indicated. Collaterals of the olivocerebellar fibres are indicated by interrupted lines on the basis of the present (Fig. 3*B*) as well as previous observations (Ito *et al.* 1966, 1970). Further explanation is in the text.

Retinal signals are transferred to the flocculus also via a mossy fibre pathway (Maekawa & Takeda, 1975). The mossy fibre activation of flocculus Purkinje cells is rather weak and is induced with a latency significantly shorter than the climbing fibre activation. The weak depression

of canal-ocular reflexes which often preceded the prominent one ascribable to the climbing fibre activation of flocculus Purkinje cells (for example, see Fig. 4*A*) could be due to the mossy fibre activation of flocculus Purkinje cells. This possibility, however, was not tested with the acute flocculotomy.

The specific retinal projections to the flocculus indicated in Fig. 7*D*, *E* and *F* are consistent with the basic scheme previously proposed (Ito, 1972, 1973); the flocculus receives climbing fibre signals from an eye concerning the stability of retinal images and thereby should improve the performance of vestibulo-ocular reflexes to that eye for accurate ocular compensation. In fact, when the optic climbing fibre pathway to the flocculus had been interrupted chronically, the horizontal canal-ocular reflex evoked in an eye by sinusoidal head rotation no longer exhibited the adaptive modification which in normal rabbits was induced by sustained visual stimulation to that eye combined with the head rotation (Ito & Miyashita, 1975).

The retinal projection to the flocculus was not demonstrated for all of the reflexes inhibited from the flocculus. For the excitatory reflex to the i-SR muscle, the preolivary pathway could not be traced beyond the ipsilateral pretectal area (Fig. 7*A*). For the inhibitory reflex to the i-IR muscle, the stimulation of the optic pathways had no effect and that of the inferior olive yielded unconclusive results (Table 1). The olivo-floccular projection drawn in Fig. 7*B* is based upon the general postulate that cerebellar Purkinje cells are all equipped with climbing fibre afferents (cf. Eccles, Ito & Szentágothai, 1967). The failure to reveal retinal projections to flocculus for these reflexes might be due to a block of retinal signals in the course to the flocculus which might be due to the anaesthesia. In fact, retinal signals affecting the reflexes to c-IO muscle often could not reach the flocculus (Table 1). Another possibility is that retinal signals are converted into inhibition at a stage prior to olivocerebellar neurones. Optic pathways to the flocculus via the inferior olive indeed contain inhibition (Maekawa & Simpson, 1973; Maekawa & Kimura, 1974).

The depression in the inhibitory reflex to the c-SO muscle following the stimulation of the optic pathways occurred only occasionally, and its relevance to the flocculus was not confirmed by the acute flocculotomy. Further, effect of the olivary stimulation upon this reflex remained inconclusive (Table 1). Nevertheless, time courses of the depression induced in this reflex by stimulation of the ipsilateral retina (Fig. 4*C*), optic chiasm and contralateral pretectal area closely resembled those for the inhibitory reflex to the i-LR muscle for which the retinal projection was successfully traced (Fig. 7*F*). The optic pathway of Fig. 7*C* was thus postulated. It should be pointed out that this optic pathway arises from the eye opposite to the target eye of the reflex. This is at variance with the basic scheme that the flocculus modifies a vestibulo-ocular reflex to an eye by referring

to climbing fibre inputs from that eye (Ito, 1972, 1973). However, as discussed above, negative results in Table 1 do not immediately exclude the possibility that the flocculus Purkinje cells influencing the reflex to the c-SO muscle receive climbing fibre input also from the target eye. Hence, the possibility remains that signals from two eyes converge onto the flocculus and modify the reflex to the c-SO muscle not only for the visual stability in its target eye but also for better coordination between the two eyes.

Specialization of flocculus Purkinje cells

Even though optic pathways to the flocculus via the inferior olive were only incompletely dissected in the sense mentioned above, the present results in Fig. 7 indicate that Purkinje cells in the flocculus are differentiated by highly specific connexions with different subgroups of relay neurones of the canal-ocular reflexes. At least five groups of Purkinje cells can be discriminated according to different connexions illustrated in Fig. 7; (1) *A*, (2) *B*, (3) *C*, (4) *D* and (5) *E–F*. The possibility remains that the 5th group is further separated into two corresponding to Fig. 7*E* and *F* respectively, if an appropriate criterion is introduced in future experiments. It is noted in Fig. 7 that relay neurones of the canal-ocular reflexes are also separated into different subgroups according to their specific connexions with certain flocculus Purkinje cells. For example, relay cells for the excitatory reflexes from the anterior canal can thus be separated into two groups affiliated to i-SR and c-IO muscles respectively (Fig. 7*A* and *D*), even though they appear to be located in the same area of the medulla (Ito *et al.* 1976*a*).

The flocculus receives primary vestibular afferents not only from the canals but also from the otolith organs (Carpenter *et al.* 1969). Vestibulo-ocular reflexes evoked by stimulation of the VIIIth nerve often contain weak responses which are not explicable as arising from canals and which therefore are ascribable to otolith organs (Ito *et al.* 1976*a*). In unpublished work by M. Ito, N. Nisimaru and M. Yamamoto such weak responses were occasionally recorded from MR and LR muscles and were depressed by stimulation of the ipsilateral flocculus and related structures, in the manner described in this article for the canal-ocular reflexes. It is probable that the flocculus contains another population of Purkinje cells specialized in connexion with otolith organs.

This work was supported by a grant from the Educational Ministry of Japan (010602).

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