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Review

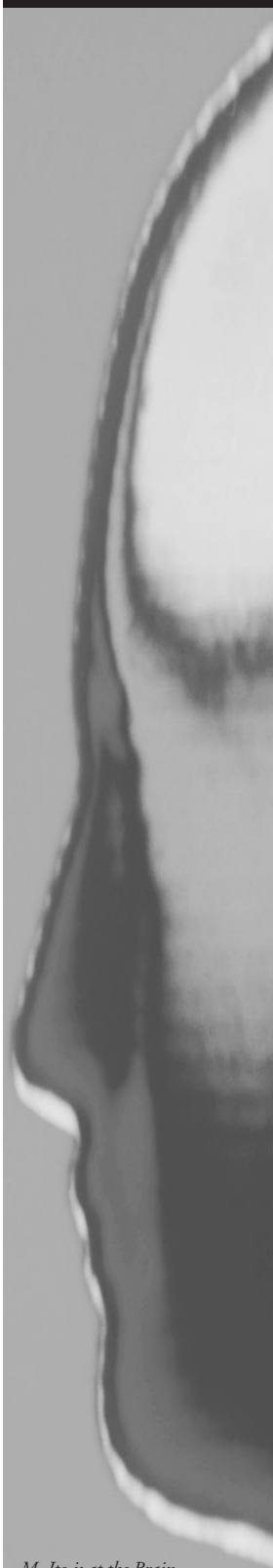
Cerebellar learning in the vestibulo–ocular reflex

Masao Ito

The vestibulo–ocular reflex, because of its close relationship with the cerebellum and its marked adaptiveness, has become a model system for studying the functions of the cerebellum. It has been hypothesized that an evolutionarily old part of the cerebellum, the flocculus, forms a modifiable accessory pathway for the vestibulo–ocular reflex arc for adaptive control, and that the modification is due to the synaptic plasticity induced by retinal errors conveyed by a unique structure of the cerebellum, the climbing fibers. The flocculus hypothesis has been supported by several lines of evidence, including lesioning or functionally impairing the flocculus and recording the activity of flocculus Purkinje cells, and, more recently, from pharmacologically or genetically inhibited synaptic plasticity, which produces long-term depression. There has also been debate on a possible site for memory retention in vestibulo–ocular-reflex adaptation, and about the signal content in flocculus Purkinje cells. This article reviews recent studies on the learning mechanisms of the cerebellum that underlie the adaptation of the vestibulo–ocular reflex.

The vestibulo–ocular reflex, commonly termed the VOR, evokes eye movements in the direction opposite to head movement, thus serving to stabilize vision automatically relative to space. Around 1930, the VOR attracted the attention of anatomists and physiologists (including Magnus[1], Lorente de No[2] and Szentágothai[3]) as a simple neural system mediated by a three-neuron arc and displaying a distinct function. In recent years, the VOR has been re-examined because of its close relationship with the cerebellum.

The VOR arc is associated with the flocculus, an evolutionarily old part of the cerebellum, and therefore provides a simple model system for studying the functions of the cerebellum. The flocculus has been proposed to be a center of learning in VOR gain[4] (Box 1). Another evolutionarily old part of the cerebellum, consisting of the nodulus and



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 1364-6613/98/$19.00 PII: S1364-6613(98)01222-4

Box 1. The flocculus hypothesis

Integrating the knowledge of neuronal circuits[a] and the marked adaptiveness of the vestibulo–ocular reflex (VOR)[b] with Marr's and Albus' learning-network theories of the cerebellum, the flocculus hypothesis was proposed around 1970 (Ref. c). The hypothesis originally ascribed three possible roles to the flocculus: (1) as an accessory path for the major VOR arc, the flocculus contributes to the dynamic characteristics of the VOR; (2) through visual feedback, the flocculus rapidly corrects the VOR in order to maintain constancy of retinal images (rapid adjustment of the VOR by vision); (3) when correction is repeated, there is a progressive change in the internal parameters of the flocculus (learning) so that performance of the VOR will be improved (adaptive modification of the VOR by vision).

The first role is evident in rabbits, which show appreciable reduction of horizontal VOR gain after flocculectomy[d]; however, flocculectomy produces no such effect in monkeys. This variability of the effects of flocculectomy can be explained based on the observations of two types of response in flocculus Purkinje cells. In response to vestibular signals from the ipsilateral labyrinth, the in-phase Purkinje cells increase their inhibitory discharges to VOR-relay neurons and thereby act to reduce the VOR gain. On the other hand, the out-of-phase Purkinje cells decrease their inhibitory discharges, and thereby act to increase the VOR gain. The flocculus of normal rabbits is dominated by the out-of-phase Purkinje cells[e], and so it is likely that the excess of out-of-phase over in-phase activity adds to the VOR gain; removal of this surplus by flocculectomy leads to a decrease in VOR gain. In monkeys, however, populations of Purkinje cells exhibiting these two types of activity are about equal in size[f] and, owing to cancellation of their effects, the flocculus will not add to the VOR gain, and flocculectomy will then produce no effect on it.

The second role is now understood as being due to the synergistic action of the optokinetic eye-movement response, which is also under the adaptive control of the flocculus. The third role, implying learning in the VOR, is the core of the flocculus hypothesis and is explained as taking place by a shift of the above-mentioned response types of flocculus Purkinje cells (see Box 3).

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uvula, has been related to the integrator function associated with the VOR, which controls VOR phase[5].

The VOR is complex, containing a number of component reflexes arising from the three semicircular canals and two otolith organs in each labyrinth, and acting on the six extraocular muscles in each eye. Under experimental conditions, the VOR is usually broken down into its components by applying yaw, pitch, roll, linear motion or static tilt to the head and measuring the horizontal, vertical or torsional movements of the eyes. However, under freely moving conditions in three-dimensional space, the VOR is mediated by the concerted operation of numerous parallel pathways linking the ten sensors in two labyrinths with the 12 muscles in two eyes[6]. This situation suggests involvement of the motor-coordination mechanisms that have been regarded as one of the characteristic features of cerebellar functions.

Among these multiple functions of the cerebellum associated with the VOR, this article will focus on the learning function of the flocculus in VOR, which has been extensively studied during the past three decades and has raised some controversial arguments.

The neuronal circuit for the VOR–flocculus system

Of the many component pathways of the VOR arc operating under the influences of the flocculus[7], that serving the horizontal VOR is illustrated in Fig. 1. The horizontal semicircular canals are stimulated by ipsilateral head rotations (as indicated by broken arrows in Fig. 1 for the left horizontal canal). The stimulated horizontal semicircular canal on either side of the head sends neural signals via the primary vestibular nerve fibers to the relay cells of the VOR located in the vestibular nuclear complex. The relay cells in turn send either excitatory or inhibitory signals to motor neurons in the abducens and oculomotor nuclei. When the head is rotated to one side, contraction of the ipsilateral medial rectus and contralateral lateral rectus muscles, and relaxation of the ipsilateral lateral rectus and contralateral medial rectus muscles are induced, so that both eyes move in the direction opposite to that of the head rotation (as indicated by solid arrows in Fig. 1) and correct the effects of head rotation on the retinal images of the external world.

In rabbits and cats, the flocculus has five to six major folia, and, in rats, usually only one. In the classic anatomy of the monkey cerebellum, ten folia have been described in the flocculus, but recent studies on neuronal connectivity have revealed that the rostral five folia belong to the ventral paraflocculus and not to the flocculus itself[8,9]. Purkinje cells in the ventral paraflocculus behave differently from those in the flocculus[10] and are related to ocular movements following movement of the visual field[11] and smooth pursuit of a moving target[12], but not with the VOR.

A proportion of Purkinje cells in the flocculus supply inhibitory synapses to both excitatory and inhibitory relay neurons in the VOR arc from the ipsilateral horizontal canal to the ipsilateral eye (Fig. 1). These Purkinje cells are localized to a narrow zone (the H zone, about 1 mm across) extending across the folia of the flocculus[13–16]. This H zone is flanked by other zones related to vertical or torsional VOR; a simple method to identify them in physiological

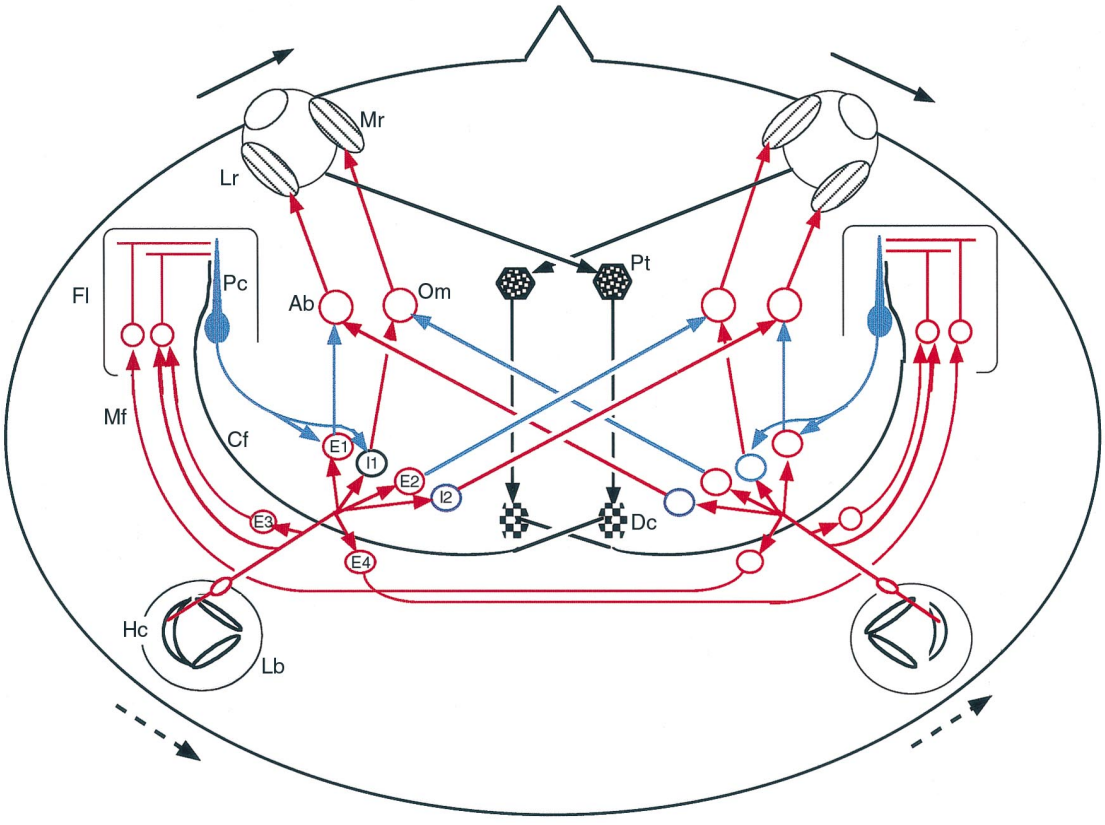

Fig. 1 Neuronal circuits for horizontal VOR. Connections from bilateral labyrinths to bilateral eyes serving for the horizontal VOR are shown, but internuclear connections between abducens and oculomotor nuclei, and commissural connections between bilateral vestibular nuclei are omitted for simplicity. Ab, abducens motor neuron; Cf, climbing fiber; Dc, dorsal cap; E1, E2, E3, E4, secondary vestibular neurons of an excitatory nature; Fl, flocculus; Hc, horizontal canal; I1, I2, secondary vestibular neurons of an inhibitory nature; Lb, labyrinth; Lr, lateral rectus muscle; Mf, mossy fiber; Mr, medial rectus muscle; Om, oculomotor neuron for Mr; Pc, Purkinje cell; Pt, pretectal region. Red lines and arrows indicate excitatory action, and blue ones inhibitory action.

experiments is the local electrical stimulation of these zones, which induces distinct horizontal, vertical or rotatory eye movements. Owing to the fine mosaic structure of the flocculus (which contains a number of microzones serving different types of VOR or even other ocular functions, such as saccade, vergence or eye blink), great caution must be exercised in relating data obtained from the flocculus without identification of the H zone to the horizontal VOR.

It has long been assumed that primary vestibular fibers arising from the semicircular canals pass directly to the flocculus on the same side as a mossy-fiber input. However, recent reexaminations have indicated that direct projections of primary vestibular fibers into the flocculus occur to a much lesser degree than previously believed in cats[17] and are probably absent in rabbits[18]. Nevertheless, vestibular ganglion cells were labeled by tracers injected into the ipsilateral monkey flocculus[9]. The field potentials elicited in rabbit flocculus by electrical stimulation of the ipsilateral vestibular nerve exhibited two peaks, apparently corresponding to the primary and secondary projections[19]. Therefore, we may assume that vestibular signals to the ipsilateral flocculus comprise both primary and secondary projections (Fig. 1), the latter arising from certain vestibular nuclear neurons[20]. (The vestibular projections to the contralateral flocculus are entirely secondary.) These vestibular projections, whether primary or secondary, terminate in the cerebellar cortex as mossy fibers, and their signals are relayed by granule cells and their axons (parallel fibers) to Purkinje cells and other interneurons in the cerebellar cortex. Electrical stimulation of the vestibular nerve of either side evokes simple spike (SS) discharges from the Purkinje cells of the flocculus, with latencies of 3–6 ms (Ref. 19).

The cerebellar cortex also receives input through a unique structure of the cerebellum, the climbing fibers (Fig. 1). The visual pathway arising from the retina and passing through the contralateral pretectal area of the brainstem reaches the dorsal cap region of the inferior olive, which projects climbing fibers across the mid-line to the flocculus[21]. Complex spikes (CSs) are elicited via this pathway from the flocculus Purkinje cells in response to retinal slips[22–24], which occur when the VOR compensates insufficiently for head movements and therefore imply errors in the VOR (retinal errors) (see Box 2).

Based on these relationships between the VOR arc and the flocculus, it has been hypothesized that the horizontal VOR is regulated at the level of its relay neurons by inhibitory signals from flocculus Purkinje cells in response to mossy-fiber-mediated horizontal-canal signals, and that these Purkinje-cell signals are modified by climbing-fiber signals representing error signals from the retina (Fig. 2A). The VOR is consequently modified, resulting in the minimization of retinal errors (Box 1). In the 1970s, evidence for synaptic plasticity in the cerebellar cortex was scarce, and the flocculus hypothesis encouraged efforts to clarify the

Box 2. Climbing fibers convey error signals

Climbing fibers are a unique, characteristic structure of the cerebellum, and numerous interpretations have been proposed for their functional role. Around 1970, it was suggested that they might be involved in 'comparison between command signals from higher centers and the activity these signals evoke at lower levels'[a], 'instruction of control errors'[b] and 'misperformance in pattern recognition'[c]. These have similar implications, in that the climbing fibers monitor the performance of a system in which a given cerebellar circuit plays a role, and in that climbing fibers inform Purkinje cells of errors caused by their misperformance.

As climbing-fiber signals evoke characteristic complex spikes in Purkinje cells, evidence in support of the above notions has been collected by observing complex spike discharges associated with various types of errors, such as occur in eye movements, posture, locomotion and hand movements. Complex spikes are associated with nociceptive signals, which are the most common error signals in motor performance[d]. In the eye-blinking conditioned reflex, complex spikes elicited by corneal stimulation would also represent errors in eye closure[e]. In short-lasting reaching-arm movements of monkeys to touch a visual target that appeared at a random location on a screen, complex spikes were found to represent two types of errors[f]. By use of information theory, it was shown that the complex spikes occurring at the beginning of movements encode the absolute destination of the movements, while those occurring at the end of the short-lasting movements encode the relative errors at the end of the reach. The former, as a movement-error signal, might help to guide the hand to the target, while the latter might help to improve subsequent movements and so to refine motor skill.

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then-unknown synaptic plasticity. An experimental counterpart of the postulated synaptic plasticity was later discovered in long-term depression (LTD) of the transmission from parallel fibers to a Purkinje cell following repeated conjunctive stimulation of the parallel fibers with a climbing fiber that innervates the same Purkinje cell[25] (Box 3). The flocculus hypothesis has thus become a basis for testing the role of LTD in cerebellar function.

The VOR and its adaptation

In laboratory experiments, the horizontal VOR is induced by sinusoidal or velocity-step head rotation, and the VOR gain is measured as the ratio of the attained eye velocity to the applied head velocity. Using sinusoidal rotation is convenient for measuring the gain and phase of the VOR separately, while velocity steps enable us to separate components of VOR responses, which arise with different latencies. The measurement of the VOR is performed in the dark or with the eyes closed, in order to avoid interference by optokinetically evoked eye movements, and by rotating the whole body instead of the head alone, in order to avoid interference by ocular reflexes elicited from the neck and trunk.

The VOR exhibits marked adaptive changes in gain under sustained mismatching between movements of the head in space and movements of the visual surroundings. Long-term visuo–vestibular mismatching for days to months can be created by the use of prism or lens goggles but, for short-term mismatching (1–4 hours), combined rotation of the turntable on which the animal is mounted and a screen representing the visual surroundings is convenient. Wearing Dove-prism goggles, which reverse the right–left axis of the visual field, and in-phase rotation of the turntable and screen in the same direction both cause adaptive reduction of the horizontal VOR gain; conversely, wearing 23 magnifying lenses and out-of-phase rotation of the turntable and screen in opposite directions both cause adaptive increase in the horizontal VOR gain. In all these situations, the VOR gain changes to reduce retinal errors.

Vertical VOR also undergoes marked adaptation[26]. A cross-modal adaptation occurs between horizontal and vertical VORs when yaw rotation on the horizontal turntable is combined with vertical optokinetic motion; the VOR measured in the dark acquires a vertical component[27].

Evidence for the flocculus hypothesis

The first lines of evidence in support of the flocculus hypothesis were derived by lesioning the flocculus or impairing its function. VOR adaptation no longer occurs after surgical ablation of, or the injection of toxic amino acids into, the flocculus in cats[28], rabbits[29,30], monkeys[31] and goldfish[32,33]. Microdialysis of lidocaine into the goldfish cerebellum blocked both adaptive increase and decrease of VOR gain[34]. Interruption of the climbing-fiber input to the flocculus reproduced the results of flocculectomy[35–37]. In addition, injection of a β-noradrenergic blocker into rabbit flocculus significantly reduced VOR adaptation, presumably by interfering with neuronal events in the flocculus[38].

The second line of evidence was collected from recordings of flocculus Purkinje cells[39–41]. The major signals of Purkinje cells are SSs, elicited by mossy-fiber inputs. In rabbits, ipsilateral horizontal head rotation either increases (in-phase type) or decreases (out-of-phase type) the SS discharges from H-zone Purkinje cells (Box 1). During sustained visuo–vestibular mismatching, the SS modulation of H-zone Purkinje cells becomes predominantly out-of-phase or in-phase, which is expected to increase or decrease the VOR gain, respectively (Box 4).

The third line of evidence supports the assumption that VOR adaptation is caused by the induction of LTD in synapses of H-zone Purkinje cells by the conjunction of rotation-induced bilateral mossy-fiber inputs and retinal-error-induced climbing-fiber inputs. This satisfactorily explains how the SS modulation of Purkinje-cell discharges changes during sustained visuo–vestibular mismatching (Box 4; Fig. 2A,C). Injection of hemoglobin into the subdural space over the flocculus abolished VOR adaptation in both rabbits and monkeys[42], presumably by the scavenging action of hemoglobin on nitric oxide (NO), which is required for the induction of LTD. The injection of an inhibitor of NO synthase into goldfish cerebellum also inhibited adaptive increase of VOR gain but, surprisingly, had no effect on the adaptive decrease in VOR gain[43]. The induction of LTD also requires the activation of protein-kinase C (PKC) and, accordingly, transgenic mice that selectively express the pseudosubstrate PKC inhibitor PKC[19–31] (a 13 amino-acid peptide corresponding to sequence 19–31 of PKC) in Purkinje cells lack VOR adaptation[44].

Debate 1: Memory site for the VOR adaptation

Although the aforementioned three lines of evidence consistently indicate that the flocculus plays a crucial role in the induction of the VOR adaptation, there has been dichotomy of opinion about the role of this structure in its retention. To determine whether the VOR adaptation is retained in the flocculus or not, the effects of the (surgical or functional) removal of flocculus functions after the VOR adaptation had developed was studied. In goldfish, no less than 30% of the altered VOR gain was retained after ablation of the cerebellum[33], favoring the view that the cerebellum is not the sole site responsible for VOR-adaptation retention. By contrast, microdialysis of lidocaine into goldfish cerebellum abolished both induction and retention of the VOR adaptation[34], suggesting that VOR adaptation is remembered, probably entirely, in the cerebellum.

Luebke and Robinson[45] used 7 Hz stimulation of the inferior olive in cats in order to suppress simple spikes from the flocculus Purkinje cells. This reversible shutdown of the flocculus prevented the induction of the VOR adaptation but did not affect the VOR gain that had been adapted; these results appear to favor the view that the VOR adaptation is not retained in the flocculus. However, one serious technical problem must be borne in mind: as Purkinje cells were silenced widely in the flocculus, the 7 Hz stimulus was probably not precisely localized to the caudal portion of the dorsal cap, the region that specifically projects climbing fibers to the narrow H zone[46]; rather, it probably targeted the neighboring ventrolateral outgrowth, projecting to the two zones flanking the H zone. Although Luebke and Robinson assumed that the H zone was involved in the shutdown[45], there was no direct evidence for this. In view of the fact that the rostrocaudal extension of the ventrolateral outgrowth and the dorsal cap stretch over nearly 3 mm, it is difficult to believe that stimulation with a single electrode targets both areas of the inferior olive. Their observation of VOR adaptation being prevented by the 7 Hz stimulus does not necessarily imply that the H zone was involved in the shutdown, because generation of climbing-fiber signals to the H zone could be disturbed by stimulation near (even if not at) the dorsal cap. It is necessary to confirm direct involvement of the H zone in the shutdown before it can be concluded that memory for VOR adaptation is controlled from outside the flocculus.

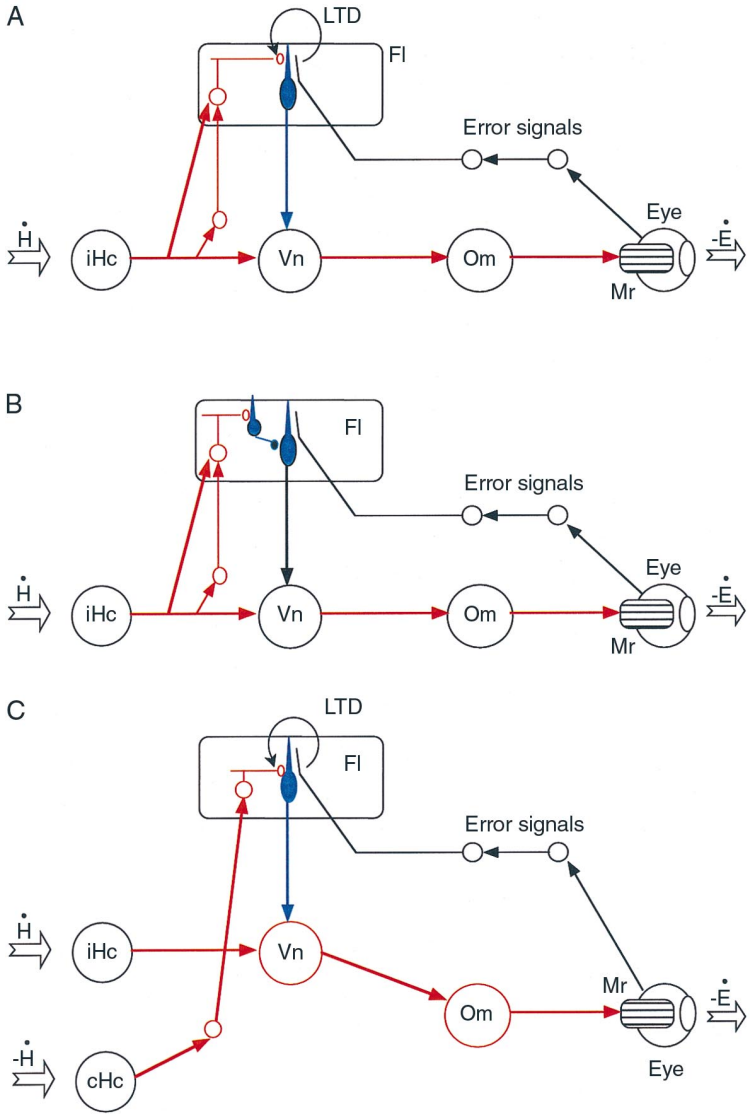

Fig. 2 Neuronal circuits for the VOR adaptation. (A) Mossy-fiber–parallel-fiber pathway from the ipsilateral horizontal canal (iHc) to the flocculus (Fl) Purkinje cells. Vn, vestibular nucleus; Om, oculomotor nucleus; Mr, medial rectus muscle. Red, excitatory cells and their processes; blue, inhibitory ones. $\dot{H}$ represents horizontal head velocity and $-\dot{E}$ horizontal eye velocity in the opposite direction to the head. **(B)** Mossy-fiber–parallel-fiber-inhibitory interneuron pathway from iHc. **(C)** Mossy-fiber–parallel-fiber pathway from the contralateral horizontal canal (cHc) to the flocculus Purkinje cells.

The Y group of vestibular nuclei mediate the vertical VOR arising from the anterior and posterior canals, and acting upon the contralateral superior rectus and inferior oblique motorneurones. As Y-group neurons in squirrel monkeys exhibit prominent changes in their responses to vertical head rotation, correlated with the adaptive gain changes in the vertical VOR, an attempt was made to determine whether these adaptive changes are mediated solely by the flocculus or not[26]. When muscimol was injected into the flocculus in order to inhibit Purkinje cells, the adapted responses of Y-group neurons were partially lost in two of the three monkeys tested, and completely in the remaining one. The partial removal of the adapted responses observed in

Box 3. Cerebellar LTD and its two forms

The original form of cerebellar long-term depression (LTD) is the persistent reduction in the transmission efficacy from parallel fibers to a Purkinje cell, which occurs after the parallel fibers have been activated repeatedly in conjunction with a climbing fiber innervating the same Purkinje cell. The requirement for the parallel-fiber–climbing-fiber conjunction for inducing LTD is consistent with the earlier assumptions adopted in Marr's and Albus' learning-network theories of the cerebellum and with the flocculus hypothesis (see Boxes 1 and 4). Since the LTD of parallel-fiber synapses was found to be induced by strong parallel-fiber stimulation alone[a,b], the role of LTD in learning has been questioned[c]. However, as climbing fibers convey error signals (see Box 2), conjunction-induced LTD is more likely to be a unique neural substrate of error-driven motor learning. The LTD induced by strong parallel-fiber stimulation may imply a local feedback to normalize excitation of Purkinje cells[c] or another type of learning, as may occur in the cerebellum-like structure of fish, which is devoid of climbing fibers[d–f]. One may suppose that these two forms of LTD share different roles in the cerebellar cortical circuitry.

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two monkeys would not necessarily imply that the adapted VOR gain is shared by both flocculus and brainstem circuits, for muscimol injection may not inactivate all flocculus Purkinje cells involved in the VOR adaptation.

Another strategy taken for determining whether the memory underlying VOR adaptation is retained in the brainstem or the cerebellum (Fig. 3A) is an analysis of the component eye movements elicited by velocity-step

Box 4. How does LTD in the flocculus account for VOR adaptation?

Purkinje cells in the flocculus H-zone exhibit two types of simple-spike (SS) modulation (in-phase and out-of-phase) in response to ipsilateral head rotation (to the side of the flocculus concerned) (see Box 1). How these two types of modulation emerge cannot be explained based on the simplified diagram of the VOR arc shown in Fig. 2A. Two more elements must be incorporated into the circuit: inhibitory neurons (stellate and/or basket cells) in the flocculus, which convert excitatory signals from the ipsilateral labyrinth into inhibitory ones (Fig. 2B), and the vestibular mossy-fiber input to the flocculus from the contralateral labyrinth, which is stimulated out-of-phase with the ipsilateral labyrinth (Fig. 2C). Thus, the Purkinje cells that receive vestibular influences predominantly through the pathway shown in Fig. 1A will exhibit the in-phase type of modulation, while those influenced predominantly through the pathway in Fig. 2B or C will exhibit the out-of-phase type of modulation.

As the in-phase type of Purkinje cell depresses, and the out-of-phase type enhances, the horizontal VOR, the shift of dominance between these response types as observed during VOR adaptation explains how flocculus Purkinje cells can either increase or decrease the VOR gain; dominance of the in-phase type leads to a decrease in the VOR gain, while dominance of the out-of-phase type leads to an increase in the VOR gain.

Complex spikes are evoked in H-zone Purkinje cells by retinal slips whenever the visual surroundings move in the temporo–nasal direction relative to the ipsilateral eye. Under the visuo–vestibular mismatching conditions that induce an increase in the horizontal VOR gain, complex spikes are elicited during the ipsilateral head rotation, which stimulates the ipsilateral labyrinth[a]. In this situation, there will be an increased probability of the complex spikes' coinciding with the vestibular mossy-fiber signals forwarded from the stimulated ispilateral labyrinth. LTD would occur in those parallel-fiber synapses receiving vestibular signals from the ipsilateral labyrinth, so that the synaptic drive causing the in-phase type of response in H-zone Purkinje cells will be reduced; the out-of-phase type of response will then dominate among H-zone Purkinje cells.

In contrast, under the visuo–vestibular mismatching conditions that induce a decrease in the horizontal VOR gain, complex spikes are evoked in H-zone Purkinje cells by the contralateral head rotation[a]. These complex spikes will coincide with the vestibular mossy-fiber signals forwarded from the contralateral labyrinth in the flocculus. LTD would occur in those parallel-fiber synapses receiving the vestibular signals from the contralateral labyrinth, as illustrated in Fig. 3C. No LTD occurs in the inhibitory synapses on Purkinje cells, as illustrated in Fig. 3C. Thus, the influence from the contralateral labyrinth to produce out-of-phase responses in the H-zone Purkinje cells will be reduced, so the in-phase type of response will dominate.

Computer simulations incorporating LTD have successfully reproduced the VOR adaptation[b,c].

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head stimuli with different latencies. In goldfish, adaptive changes occurred in the earliest component of the eye movement elicited, with a latency of 18 ms; if this component is induced via the three-neuron VOR arc, the results favor the brainstem memory site[30]. A similar suggestion was also made in monkeys, in which the early response (which has a latency of 13 ms) was adaptively modified[47]. However, an earlier response before the 13 ms latency was not modified, indicating that the shortest-latency VOR pathways are not modified[48]. When electrical stimulation of a horizontal canal with implanted electrodes was adopted to induce early and late eye-velocity changes[49], the early component (with a latency of 5–15 ms), probably mediated by the three-neuron VOR pathway, showed a substantial change correlated with adaptive VOR-gain changes in only one of the four monkeys tested, and only a small or negative change in the other three. By contrast, the late component (with a latency of 20–45 ms) exhibited large changes correlated with adaptive VOR-gain changes in all of the four monkeys. In monkeys, therefore, it is unlikely that the VOR adaptation is retained in the rapidly transmitting three-neuron arc, but the question of whether the late, modifiable responses are mediated in the flocculus, the brainstem or both (as asserted recently[50]) still remains unsolved.

Debate 2: Purkinje-cell behavior associated with VOR adaptation

Another debate concerns the behavior of flocculus Purkinje cells. Even though this behavior conforms to the flocculus hypothesis in the rabbit H zone (Boxes 1 and 4), inconsistent results have been reported in monkey and goldfish experiments. However, these experiments involve serious technical problems: the earlier data on monkeys were obtained without distinguishing between the ventral paraflocculus and the flocculus[8]. It is also questionable whether the vestibular responses of Purkinje cells can be measured during the visual suppression of VOR[51]; visual suppression eliminates oculomotor signals but could introduce another set of neural signals to cancel the VOR. When this component, presumably arising from the smooth-pursuit eye-movement system, is ignored, the visual-suppression method could overestimate the intensity of vestibular responses of Purkinje cells. In goldfish, cancellation of the VOR by optokinetic stimulation was adopted when evaluating vestibular signals[52]; the behavior of Purkinje cells so observed would represent a combination of vestibular and optokinetic responses, and cannot be related uniquely to the VOR adaptation.

Debate 3: Implications of oculomotor signals

In the aforementioned visual-suppression method, the difference between the responses during VOR and those during visual suppression was considered to represent oculomotor signals encoding eye velocity or eye position. As the oculomotor signals so derived were large, it has been hypothesized that oculomotor signals provide positive feedback from the oculomotor system to the VOR-relay neurons via the flocculus[53] (Fig. 3B). If this were the case, changes in Purkinje-cell behavior observed during VOR adaptation could reflect changes in eye movements resulting from adaptation and would not be the cause of adaptation, as postulated in the flocculus hypothesis.

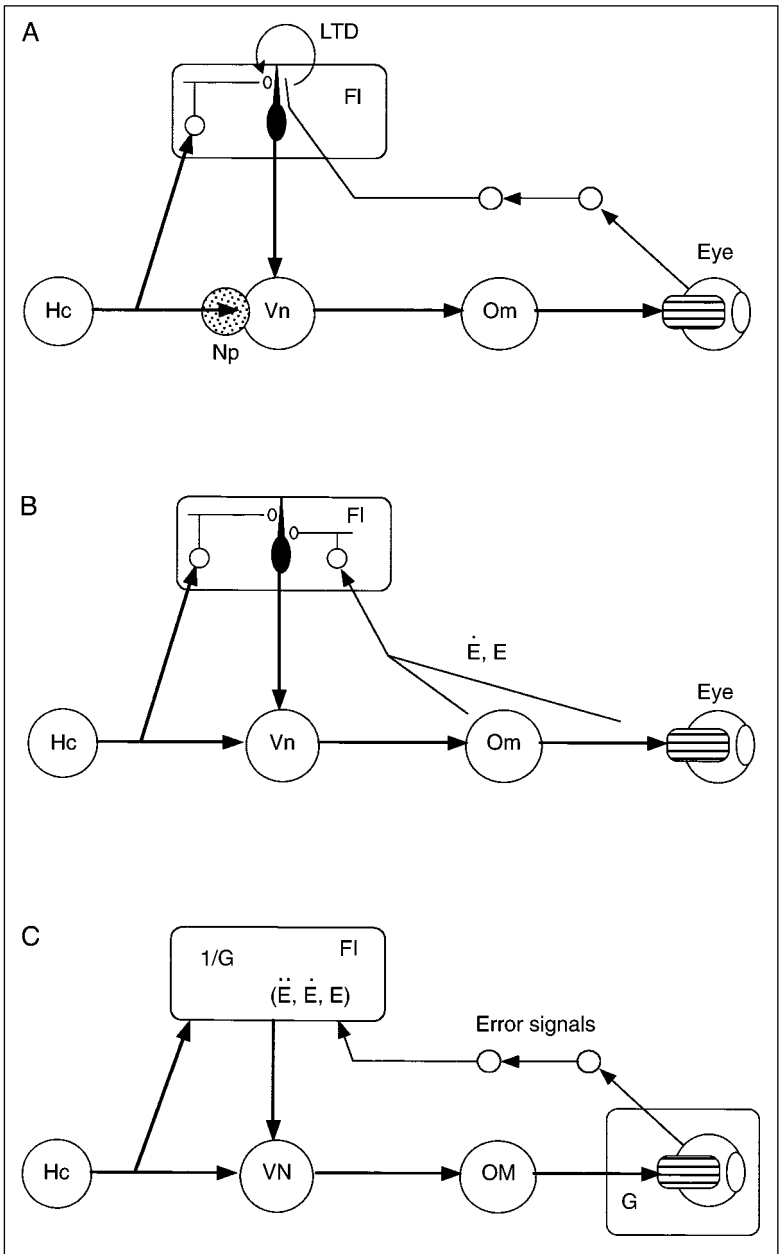

Fig. 3 Various models of VOR adaptation. (A) Multiple-memory-site model. Synaptic plasticity is assumed to be present in the brainstem, most likely in the vestibular nucleus (Np). (Other abbreviations as Fig. 2.) **(B)** Positive feedback of oculomotor signals. E represents eye position; Ė, eye velocity. **(C)** Inverse-dynamic model in the flocculus. G represents the dynamics of the eyeballs; 1/G, the inverse of G; Ë, eye acceleration.

Spontaneous eye movements in the dark are accompanied by Purkinje-cell responses in cat flocculus, which might represent oculomotor signals[54]. In rabbits, however, SS responses to head rotation and screen rotation observed in H-zone Purkinje cells are distributed independently of each other, suggesting that these responses do not contain oculomotor signals, as these would be common to both responses if this hypothesis were true[55]. Weak electrical stimulation of the optic tract induces nystagmus and after-nystagmus in the dark, and also concomitant SS responses in H-zone Purkinje cells. As the blockage of proprioceptive afferents from extraocular muscles eliminated about a third of the SS responses, the optic-tract-induced responses in Purkinje

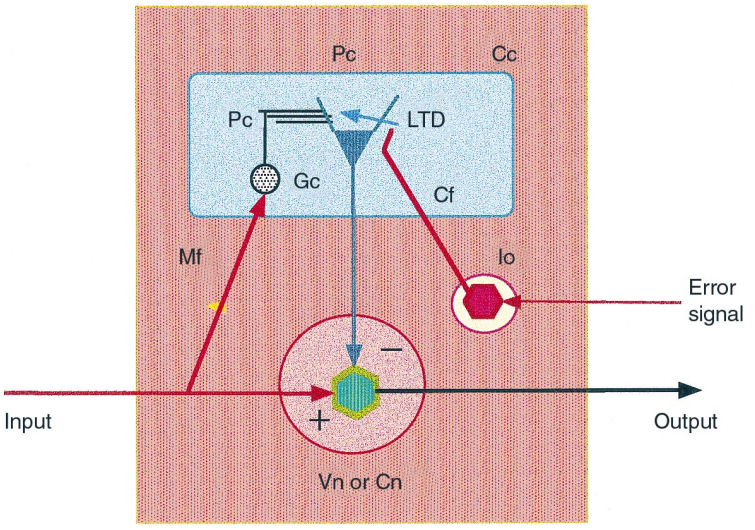

Fig. 4 Cerebellar corticonuclear microcomplex. The microcomplex consists of a small area of the cerebellar cortex (Cc) along with a small group of nuclear neurons that receive Purkinje-cell (Pc) inhibition from the former. Mossy-fiber (Mf) signals provide inputs to the microcomplex, while the nuclear neurons generate outputs of the microcomplex. Climbing-fiber signals represent errors and act to reorganize internal connections in the microcomplex, leading to modification of its input–output relationship[60]. LTD, long-term depression; Cf, climbing fiber; Cn, cerebellar nucleus; Io, inferior olive; Gc, granule cell; Pf, parallel fiber; Vn, vestibular nucleus.

cells may represent, in part, oculomotor signals from extraocular receptors and, in part, those generated in a brainstem neuronal circuit[56]. In a similar experiment, eye-velocity responses were seen in most H-zone Purkinje cells tested, and these accounted for 20% of their turntable-rotation-induced responses, while eye-position responses were seen in one third of these cells and accounted for 14% of their turntable-induced responses[57]. These relatively small percentages could still be an overestimate of oculomotor signals, if optic-tract stimulation does induce any optokinetic signals. In rabbits, therefore, positive feedback of oculomotor signals via the flocculus does not seem to be a prominent feature of VOR control, even if it were to exist.

Another interpretation of the eye-movement-related Purkinje-cell responses arises from a control-system view of the VOR. Optimal operation of a feedforward system like the VOR requires the dynamics of the controller to be inversely proportional to the dynamics of the controlled object[58]. Therefore, the behavior of Purkinje cells in the flocculus H zone, as part of the controller for the VOR, would reflect the inverse dynamics of the eyeballs (Fig. 3C). No investigation on the flocculus has been performed in this regard but, even though eye movements are elicited by movements of the visual field, Purkinje cells in the ventral paraflocculus have been shown to exhibit SS modulation corresponding to component terms of eye acceleration and velocity in the equation describing the inverse dynamics of the eyeballs[11]. A hypothesis still to be tested is whether flocculus-Purkinje-cell behavior reflects eye movements as part of the inverse dynamics of the eyeballs computed within the flocculus, so as to cause VOR adaptation, and not feedback from the moving eyes. Experimental testing is needed to determine which of these two possibilities is, in fact, the case.

Outstanding questions

- Does LTD account for long-term or even permanent memory? This has not been answered because of technical difficulties that currently do not allow us to follow the time course of LTD for more than three hours. This question is central to the cerebellar-learning theories and, to answer it, new technologies for marking LTD are required.
- Is the memory in VOR adaptation controlled by the flocculus alone or by brainstem pathways, or by both of them? Although the available evidence consistently indicates that the flocculus, with the LTD mechanisms, plays a crucial role in induction of the VOR adaptation, a role of the flocculus in its retention still remains to be verified. If a brainstem pathway is involved, how can the adaptation induced in the flocculus be transferred to the brainstem pathway (see Fig. 3A)?
- How can the functional role of oculomotor signals in VOR be determined? The eye velocity and position signals in flocculus Purkinje cells might reflect feedback from the oculomotor system to the flocculus, or it might encode the inverse dynamics of eyeballs. How might these two alternative possibilities be distinguished experimentally?

Conclusion

The VOR–flocculus system conforms to the general idea of a cerebellar corticonuclear microcomplex as a module capable of error-driven learning to modify its input–output relationships[59,60]. Controversies about the neuronal mechanisms in a microcomplex need to be resolved in future but, as the microcomplex includes both a cerebellar cortical zone and a nuclear cell group (Fig. 4), the debate on the memory site for VOR adaptation is not critical for the microcomplex concept. Such microcomplexes have a role in various systems of the brain and spinal cord, and play diverse roles in central-nervous-system functions, from reflexes up to cognitive functions. The VOR–flocculus system as a microcomplex merits further investigation to form an important basis for formulating a general theory to explain these diverse roles of the cerebellum.

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