after similar time periods also showed evidence of graft survival (two of the three monkeys studied had surviving grafts). Very careful observation of the grafted monkeys failed to reveal any neurological, infectious, or other problem. We believe that the initial failure to achieve graft survival in one of the monkeys was due to early difficulties in tissue collection and cryopreservation methods that have been eliminated with subsequent efforts. All animal work was carried out according to *The NIH Guide for the Care and Use of Animals* at the St. Kitts Biomedical Research Foundation, which maintains an assurance of compliance with these guidelines with the Office for Protection from Research Risks, U.S. Public Health Service.

17. In an earlier attempt at cross-species transplantation, we found that cryopreserved rat fetal mesencephalic cells placed into the striatum of a monkey had failed or been rejected, consistent with work previously cited in which human fetal cells were implanted into rodents (*10*). In those studies, immunosuppression with cyclosporine was effective in preventing or reducing rejection. In the first documented transplantation of human embryonic brain tissue, two Yale researchers found that tissue implanted into the anterior chamber of the eye in guinea pigs survived without rejection for up to 2 years in 50 of 55 attempts, without immunosuppression [H. S. N. Greene and H. Arnold, *J. Neurosurg.* **2**, 315 (1945)]. It is not clear, therefore, whether immunosuppression would have been necessary. Further studies of the effects of immunosuppression on transplants of human fetal tissue into the brains of monkey would be of scientific interest, but might not be predictive of the need for immunosuppression of human fetal tissue allografts implanted into human brains.
18. Previously reported fetal neural grafts in St. Kitts green monkeys (*Cercopithecus aethiops sabaeus*) may have had reduced immunogenicity due to decreased genetic heterogeneity in the St. Kitts monkey population, which is descended from a relatively small number of individuals imported from Africa 200 to 300 years ago [F. E. Poirer, *Folia Primatol.* **17**, 20 (1972)]. Whether the extent of this heterogeneity might affect neural tissue compatibility factors is not known. Successful transplants into rhesus monkeys from more heterogenous populations have been reported in a preliminary fashion [R. A. E. Bakay and F. A. King, *Lancet* **ii**, 163 (1986); K. S. Bankiewicz, D. M. Jacobowitz, R. J. Plunkett, E. H. Oldfield, I. J. Kopin, *Soc. Neurosci. Abstr.* **13**, 163 (1987); K. S. Bankiewicz *et al.*, *Soc. Neurosci. Abstr.* **14** (1), 3 (1988)]. One report has suggested that a second transplant of fetal tissue in a bonnet monkey induced rejection in an earlier graft on the contralateral side [C. Freed, J. B. Richards, K. E. Sabol, M. L. Reite, in *Pharmacology and Functional Regulation of Dopaminergic Neurons*, P. M. Heart, G. Woodruff, D. M. Jackson, Eds. (Macmillan, New York, in press)].
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Brain Stem Neurons in Modified Pathways for Motor Learning in the Primate Vestibulo-Ocular Reflex

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The vestibulo-ocular reflex (VOR) stabilizes retinal images by generating smooth eye movements that are equal in amplitude and opposite in direction to head turns. Whenever image motion occurs persistently during head turns, the VOR undergoes motor learning; as a result image stability is gradually restored. A group of brain stem neurons that are in the modified pathways has now been described. The neurons express changes in firing in association with motor learning in the VOR and receive monosynaptic inhibition from the flocculus of the cerebellum. The changes in firing have an appropriate magnitude and are expressed at the correct latency to account for the altered VOR. The response properties of the neurons point to their brain stem vestibular inputs for further investigation of the site of motor learning.

MOTOR LEARNING PLAYS A CRUcial role in establishing and maintaining the excellent performance of the vestibulo-ocular reflex (VOR). Normally, the VOR generates smooth eye movements that are equal in amplitude and opposite in direction to head movement (*1*). As a result, the eyes are stabilized in space and the retinal images of the surroundings remain stable during head turns. Any deterioration in the performance of the VOR causes retinal image motion during each head turn. The combination of visual and vestibular inputs causes learning, which, over a time course of several days, restores the performance of the VOR so that image stability is reestablished (*2*).

Although the site of motor learning has not been located, much is known about the neural basis for motor learning in the VOR. The VOR is subserved by at least two parallel pathways; only some of those pathways are subject to modification (*3, 4*). The cerebellar flocculus must be intact for motor learning to occur (*5*). The output from the flocculus changes in association with motor learning (*6*).

To investigate the site of learning we have identified neurons that receive monosynaptic inputs from the flocculus and analyzed their firing during the VOR before and after motor learning. Seven rhesus monkeys were trained to fixate and track a small, movable target. They were then anesthesized with Halothane, and sterile procedures were used to prepare each monkey for monitoring eye movements and for chronic single unit recording (*7*). We recorded from the brain stem in five of the monkeys and from the flocculus in the other two monkeys. Before beginning brain stem recordings, we cemented stimulating electrodes in the flocculus at a site where stimulation evoked smooth eye movement toward the side of the stimulated flocculus.

Motor learning was induced by fitting monkeys with spectacles that provided magnified (2.2×) or miniaturized (0.25×) vision (*4*). These spectacles require large changes in the amplitude of the VOR to restore image stability during head turns. Monkeys wore the spectacles in their home cages and underwent motor learning during active head turns. The performance of the VOR was then measured by imposing passive head rotation in the dark and computing the gain of the VOR, defined as smooth eye speed divided by head speed. Before motor learning, the gain of the VOR was between 0.9 and 1.0. We began recordings from adapted monkeys after they had worn the spectacles for 1 week, when the gain of the VOR had increased to values above 1.5 or decreased to values below 0.4.

We identified flocculus target neurons (FTNs) by the fact that they were completely inhibited for 10 to 20 ms after stimulation of the flocculus (Fig. 1A). The latency of inhibition, estimated by superimposing sweeps on an oscilloscope, ranged from 1.0 to 1.9 ms. FTNs discharged in relation to the VOR, smooth pursuit, saccades, and steady fixations (*8*). Before motor learning, their firing was modulated by head rotation when the eyes were driven by the VOR, but not when the monkey kept his eyes stationary in the orbit by tracking a target that moved exactly with his head (*9*). Although we found FTNs that preferred upward, downward, ipsilateral, or contralateral eye movements, we report only on those that increased their firing for eye movements toward the side contralateral to the recording, since they formed the majority of our sample. Reconstruction of electrode pene-

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trations and marking lesions revealed that FTNs were encountered in and near the medial vestibular nucleus ipsilateral to the stimulated flocculus, in the area that receives projections from the flocculus (*10*).

Before motor learning, FTNs showed the same relation between firing rate and eye velocity during the VOR as during pursuit with the head fixed. For example, Fig. 1 shows averages of firing rate for one FTN that was recorded in the right vestibular nucleus before motor learning. We measured firing rate during the VOR in the dark (*11*) by imposing rapid changes in head velocity from 0° to 30° per second rightward (Fig. 1C) or leftward (Fig. 1D). We measured the firing rate during pursuit by having the monkey track the sinusoidal motion of a small target with the head stationary (Fig. 1B). In both conditions, firing increased during leftward eye movement and decreased during rightward eye movement.

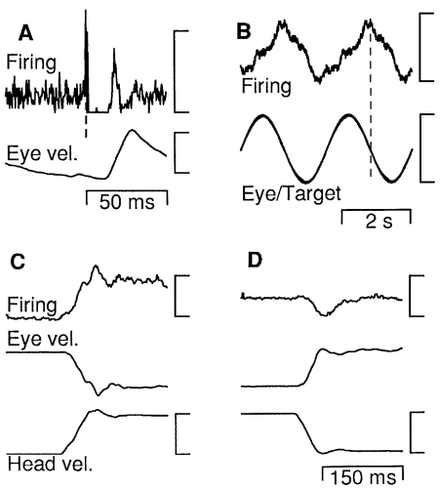

Fig. 1. Response properties of a typical flocculus target neuron. (**A**) Peristimulus time histogram accumulated from applying 200 single shocks to the flocculus while recording from this FTN. The histogram is centered on the time of the stimulus, which is indicated by the vertical dashed line. There are a large number of events in the bins immediately after the stimulus because the window discriminator was triggered by the stimulus artifact and the subsequent field potential. The lower record shows the twitch of eye velocity evoked by the stimulus. (**B**) Average firing rate during pursuit of sinusoidal target motion at 0.4 Hz, ±10°, accumulated from ten consecutive cycles of tracking. The lower records show the position of the eye and target; the vertical dashed line indicates peak eye velocity toward the side contralateral to the recording. A single cycle has been repeated to facilitate viewing of the periodic events. (**C** and **D**) Average firing rate during the VOR accumulated from at least 50 repetitions of the rapid change in head velocity toward the side ipsilateral (C) or contralateral (D) to the side of the recording. The firing rate calibrations are 400, 80, 40, and 40 action potentials per second in (A), (B), (C), and (D), respectively; the eye movement calibrations are 2° per second in (A), 20° per second in (B), and 30° per second in (C) and (D).

After motor learning, FTNs showed large changes in their firing during the VOR. Figure 2 illustrates the responses of two FTNs recorded in the right vestibular nucleus after motor learning. Both showed increased firing during leftward pursuit eye movements with the head fixed. During the leftward VOR induced by rightward head motions, the FTN recorded when the gain of the VOR was high (Fig. 2A) showed increased firing. In contrast, the FTN recorded when the gain of the VOR was low (Fig. 2B) showed decreased firing during a leftward VOR. Head motion in the opposite (leftward) direction had a reciprocal effect on firing rate in each neuron (not illustrated).

We computed the sensitivity to head velocity during the VOR as the change in firing rate divided by the change in head velocity in the first 50 ms after the onset of the rapid change in head velocity. For 14 FTNs recorded after the gain of the VOR had been increased, the sensitivity to head velocity ranged from 0.92 to 8.3 and averaged 4.3 action potentials per second per degree per second. For 13 FTNs recorded after the gain of the VOR had been decreased, sensitivity to head velocity ranged from −0.87 to +0.36 and averaged −0.47 action potentials per second per degree per second. The sensitivity to head velocity was negative in 12 of the 13 FTNs we recorded after decreases in the gain of the VOR, indicating that they had reversed the polarity of their responses to vestibular stimuli.

An earlier study revealed that not all VOR pathways are modified during motor learning. The modified pathways have a total latency from head movement to eye movement of 19 ms (*3*). We next determined whether the latency of FTN firing during rapid changes in head velocity is appropriate for neurons in the modified pathways. The histogram in Fig. 3A summarizes the latency from the onset of head motion to the first change in firing rate for 21 FTNs that were studied during rapid changes in head velocity before motor learning or after the gain of the VOR had been increased (*12*). The vertical dashed line in Fig. 3A shows the criterion latency at which FTNs would have to respond to vestibular stimulation if they contributed to the VOR 19 ms after the onset of head motion (the latency of the modified pathways). The criterion latency was estimated as 12 ms by assuming that FTN firing would affect eye movement after a latency of 7 ms [12 + 7 = 19 ms; 1 ms for FTN firing to affect motoneuron firing (*13*) and 6 ms for motoneuron firing to affect eye velocity (*14*)]. Since their latencies are grouped around the criterion latency, with a mean latency of 14.9 ms, FTNs respond to rapid changes in head velocity at the correct latency to be part of the modified pathways.

We next asked whether the changes in FTN firing rate after motor learning could result from changes in their inputs from the flocculus. This seems unlikely a priori because Miles *et al.* (*6*) recorded from Purkinje cells (PCs) in the flocculus and failed to find changes in vestibular inputs that were appropriate to cause motor learning. However, a site of motor learning outside the flocculus could affect PC firing through eye movement inputs to the flocculus.

We think that the horizontal gaze velocity PCs (*15*) are the relevant PCs for the horizontal VOR because they fulfill two of the criteria used by Watanabe (*6*) to identify the

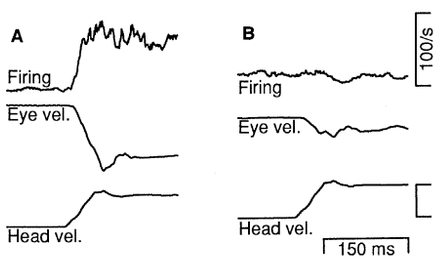

Fig. 2. Responses of two representative FTNs during the VOR after increases (**A**) or decreases (**B**) in the gain of the VOR. Both panels show the average firing rate during the VOR evoked by rapid change in head velocity toward the side of the recording. The difference in the gain of the VOR can be seen by noting the difference in the size of the eye velocity responses. The velocity calibration is 30° per second.

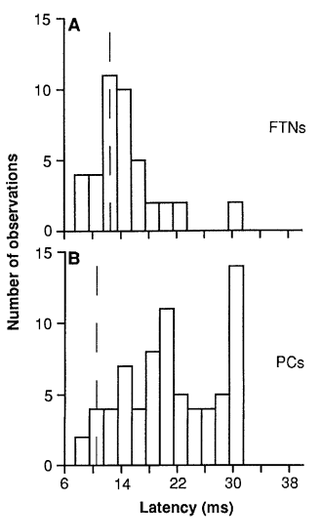

Fig. 3. Response latencies for FTNs and PCs. The histograms contain two observations per cell, one each for the responses to rightward and leftward head motion. The vertical dashed lines indicate the criterion latency at which each group of cells would have to respond to a vestibular stimulus to affect eye movement 19 ms after the onset of head motion. Data in (**A**) are from three monkeys; data in (**B**) are from two monkeys.

PCs that are important for the horizontal VOR. (i) They exhibit modulation of complex-spike firing in relation to retinal image motion away from the side of the recording (*16*). (ii) Motor learning causes their simple-spike firing to express changes in firing that are in the correct direction to support the altered VOR (*17*). The simple spike firing of these PCs is modulated preferentially for horizontal rather than vertical pursuit eye movements.

For 34 PCs studied after decreases in the gain of the VOR (*18*), the response latency during the VOR averaged 21.3 ms. The criterion latency for PCs (vertical dashed line in Fig. 3B) was estimated as 10 ms, which is 2 ms shorter than that for FTNs. This takes account of the latency at which stimulation of the flocculus inhibits FTNs (1 to 2 ms) and of the latency for evoking an eye movement by single shock stimulation of the flocculus (9 to 10 ms) (Fig. 1A). Most PCs responded too late either to contribute to the modified pathways or to cause the modified responses in FTNs (Fig. 3B). However, a few PCs did respond early enough to cause the change in FTN firing. Further experiments will be needed to test the possibility that these few PCs cause motor learning and that the FTNs and the rest of the PCs follow.

FTNs satisfy two criteria established by our earlier work for neurons in the modified pathways. One criterion is based on the fact that unmodified VOR pathways make a fixed contribution to the VOR. Changes in firing in the modified pathways will therefore have to exceed the change in the VOR. FTNs satisfy this criterion: after decreases in the gain of the VOR, eye motion is still opposite in direction to head motion, but FTNs show a reversed response. FTNs also satisfy a second, latency criterion (*3*): they respond to vestibular stimuli at a latency that is appropriate to be part of the modified pathways. We conclude that FTNs are in the modified VOR pathways. Because FTNs generally respond before PCs, future experiments should evaluate brain stem vestibular inputs to FTNs as a possible site of motor learning.

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7. Our methods are detailed in (*4*) and in S. G. Lisberger and F. A. Miles [*J. Neurophysiol.* **43**, 1725 (1980)].
8. The relation between firing rate and eye position almost always had a "knee" such that the slope was not zero only for eye positions to one side of straight ahead gaze.
9. Motor learning caused many FTNs to become strongly modulated when the monkey tracked a target that moved exactly with him during head rotation. When the gain of the VOR was low, FTNs showed increased firing during head motion away from the side of the recording. When the gain of the VOR was high, many FTNs showed increased firing during head motion toward the side of the recording.
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11. Details of the method for converting the digital train of action potentials into an analog record related to firing rate are in (*4*).
12. It would have been difficult to measure response latency from FTNs recorded when the gain of the VOR was low, because the responses were too small to allow accurate estimates of latency.
13. We are assuming that FTNs are in VOR pathways and project to motoneurons because (i) FTNs are inhibited by stimulation of the flocculus and (ii) studies in nonprimate species have shown that the flocculus inhibits direct brain stem VOR pathways [S. M. Highstein, *Exp. Brain Res.* **17**, 301 (1973); M. Ito, N. Nisimaru, M. Yamamoto, *ibid.* **24**, 257 (1976); W. Precht and R. Baker, *ibid.* **14**, 158 (1972)].
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17. Our recordings during rapid changes in head velocity agree with previous data (*6*).
18. It was not practical to measure PC response latency before motor learning, because PC firing rate normally shows very little modulation of firing rate during the VOR.

19. We thank D. Belknap, F. Miles, M. Stryker, and R. Nicoll for helpful criticism of earlier versions of the manuscript. Supported by EY03878 from the National Institutes of Health, BNS 8444605 from the National Science Foundation, a Scholars Award from the McKnight Foundation, and a Development Award from the McKnight Neuroscience Endowment Fund.

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Spatially Resolved Calcium Dynamics of Mammalian Purkinje Cells in Cerebellar Slice

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Microfluorometric imaging was used to study the correlation of intracellular calcium concentration with voltage-dependent electrical activity in guinea pig cerebellar Purkinje cells. The spatiotemporal dynamics of intracellular calcium concentration are demonstrated during spontaneous and evoked activity. The results are in agreement with hypotheses of dendritic segregation of calcium conductances suggested by electrophysiological experiments. These in vitro slice fluorescence imaging methods are applicable to a wide range of problems in central nervous system biochemical and electrophysiological functions.

THE SPATIAL DISTRIBUTION OF IONIC channels over the plasmalemma and the associated compartmentalization of the integrative and the cell biological properties is a critical issue in the characterization of central neuronal function. The locus specificity of the synaptic input may influence both the electrical integrative properties of the cell and the degree of precision with which different compartments may be regulated biochemically. For example, the spatial distribution of second messenger systems activated by Ca^{2+} will be determined by ionic channel distribution.

The nature of the distribution of voltage-dependent ionic channels over the somatic and dendritic membranes of neurons has been investigated often in recent years (*1*). Segregation of the voltage-dependent Na^+ and Ca^{2+} ionic conductances was hypothesized when intradendritic recordings from Purkinje cells in avian cerebellum (*2*) and mammalian cerebellar slices in vitro (*3*) demonstrated the presence of voltage-dependent Ca^{2+} conductances capable of generating dendritic spikes. The results from such studies suggested that in this cell Ca^{2+} conductance is most prominent, if not exclusively present, in the dendritic tree, and the voltage-dependent Na^+ conductance is restricted to the soma and axon (*3*). Furthermore, electrophysiological examination of the slow Ca^{2+}-dependent potentials and regenerative spikes (*3*) suggested that the ionic conductances underlying these two distinct components might also be spatially separated within the dendritic tree. The experiments presented here were designed

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