response was larger after the irradiation. This increase in response amplitude is due to the increase in input resistance, as discussed above. Conversely, the response at wind position 2 was eliminated. According to the anatomical observations wind from position 2 should activate only dendrite Y. Note that no action potentials were visible in the responses recorded after the laser ablation. This indicates that the spike-initiating zone in 10-3 was located beyond the targeted region of the neurite (*15*). All the data shown in Fig. 2 were obtained from a single experiment. The experiment was repeated four times with similar results.

It appears that this dendrite does have a distinct directional sensitivity. Thus the responses of each of the three dendrites may summate to produce the overall directional sensitivity of the interneuron. By performing similar experiments in which the laser beam was aimed at different parts of the dendritic tree of neuron 10-3, we have greatly refined our knowledge about the relation between neuronal form and function in this system. The response properties of this sensory interneuron can now be interpreted in terms of synaptic interactions at single dendritic branches.

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12. The tips of the electrodes were filled by syringe with 3 percent 6-carboxyfluorescein (Kodak) and the shanks were filled with 2*M* potassium acetate. The electrodes were beveled to optimum resistances (20 to 40 megohms).
13. The cricket was mounted on a stationary platform with the cerci intact and the terminal ganglion exposed for intracellular recording. All recordings were made in the soma of this neuron by using a single electrode clamp in the current clamp mode. Filiform hairs were stimulated with a jet of wind from a laminar nozzle that could be directed toward the animal from any angle in the horizontal plane.
14. The cell's response was quantified by measuring the area under the waveform for the first 100 msec during the wind puff. The maximum response value for each curve was set at 1.0 and all responses were scaled with respect to that maximum. Each value is plotted in polar coordinates according to stimulus orientation with respect to the animal's body.
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Selective Extraction of Small and Large Molecules from the Cerebrospinal Fluid by Purkinje Neurons

Abstract. *Cerebellar Purkinje neurons accumulated propidium iodide, granular blue, and horseradish peroxidase conjugated to wheat germ agglutinin but not unconjugated horseradish peroxidase, bisbenzimide, or Evans blue when these compounds were infused into the lateral cerebral ventricles of awake, unrestrained rats. Accumulation of propidium iodide by Purkinje neurons of the vermis was associated with a reproducible behavioral abnormality characterized by truncal tremor, ataxia, and nystagmus. Both the accumulation of propidium iodide in Purkinje cells and the behavioral abnormality were prevented by prior intracerebroventricular administration of ouabain or colchicine, drugs that block neuronal transport processes. The ability of cerebellar Purkinje neurons to extract small and large molecules from the cerebrospinal fluid has important implications for their physiology and pathology.*

Proteins injected into the lateral cerebral ventricles can enter brain parenchyma along the perivascular spaces of blood vessels (*1*) and can then be pinocytosed by neurons and glia (*2*). In a recent study certain plant lectins were accumulated from the cerebrospinal fluid (CSF) by Purkinje neurons of neonatal mice, while no uptake was observed in mature mice (*3*). However, the Purkinje neurons in the adult human brain have been found to contain immunologically recognizable light-chain proteins that are also present in the CSF (*4*). The possibility that Purkinje neurons can extract and accumulate molecules from the CSF could have important physiological and pathological consequences, since these neurons have large dendritic arrays in close proximity to the CSF. In this study we examined the accumulation by rat brain Purkinje cells of two proteins, horseradish peroxidase (HRP) and HRP conjugated to wheat germ agglutinin (HRP-WGA), and of four fluorescent dyes, propidium iodide (PI), granular blue, bisbenzimide, and Evans blue (*5*) after their intracerebroventricular injection. Since PI can enter the CSF after intravenous administration (*6*), we also examined the accumulation of PI by Purkinje cells after its intravenous administration.

Adult male Sprague-Dawley rats (300 to 350 g) were anesthetized and implanted with a 23-gauge metal cannula in the lateral cerebral ventricle. Five to 14 days later the animals received (without anesthesia) a unilateral intraventricular injection of 10 μl of a test substance. The compounds injected were HRP (10 percent; $n = 3$), HRP-WGA (1 percent; $n = 3$), PI (0.1 to 0.5 percent; $n = 7$), granular blue (1 percent; $n = 2$), bisbenzimide (5 percent; $n = 2$), and Evans blue (2 percent; $n = 3$). Behavioral responses were observed during the injection and postinjection periods. At various periods after the injections each rat was anesthetized with pentobarbital (60 mg/kg) and perfused with a fixative (*7*) for 20 minutes. The fixative was then washed out by an additional perfusion with 0.1*M* sodium phosphate buffer containing sucrose (15 percent, weight to volume). Brain sections were cut on a freezing microtome. Sections from rats that had received injections of fluorescent dyes were mounted and examined under a Leitz fluorescent microscope. Sections from rats that had received HRP or HRP-WGA were treated with 3,3′,5,5′-tetramethylbenzidine before mounting (*8*).

No behavioral abnormalities were noted during or after intraventricular infusion of HRP, HRP-WGA, bisbenzimide, or Evans blue. There was a brief burst of grooming behavior during the infusion of granular blue, but the rats behaved normally during the subsequent 4 hours. PI caused a reproducible behavioral abnormality, beginning at the end of the infusion period, which was characterized by an oscillating rotatory tremor of the head and body and prominent rotatory nystagmus. No tremor was present in the limbs. By 10 minutes after onset the rats

did not walk about the cage but leaned against the side with limbs outstretched and paws tightly gripping the cage bottom as though to reduce the tremor. Moving the rats away from support or changing their head and body position intensified the tremor. Bladder control and accurate paw placing were preserved, however, and tail pinching continued to provoke cries. With concentrations of PI less than 0.2 percent the abnormal behavior persisted for approximately 1 hour. Thereafter the rats behaved normally throughout a 2-week period of observation.

Cerebellar uptake of the test substances was assessed up to 4 hours after infusion (Figs. 1 and 2). At this time all the substances were found in the outer molecular layer of the cerebellar vermis [especially lobules I, II, III, VIII, IX, and X (*9*)] and in the superficial folia of the hemispheres. The outer molecular layer in deep sulci was often unlabeled, presumably because of a restricted flow of CSF into these sulci. Despite their presence in the outer molecular layer, HRP and Evans blue were not found in the somata of Purkinje neurons. Bisbenzimide diffused freely into the cerebellum and unselectively labeled the nuclei of giant cells, endothelial cells, granule cells, and some Purkinje neurons. Granular blue, HRP-WGA, and PI were concentrated in the dendrites and cell bodies of Purkinje neurons in lobules I, II, VIII, IX, and X of the vermis and in the superficial folia of the hemispheres. Purkinje neurons in deep sulci, which lacked staining in the outer molecular layer, were unlabeled. These results suggest that Purkinje neurons can selectively accumulate certain small and large molecules from the CSF and that substances injected into the CSF of the lateral ventricle do not have equal access to all parts of the cerebellum.

Since PI was readily accumulated by Purkinje neurons and caused a striking abnormality of postural control, we attempted to correlate the anatomical distribution of PI with the behavioral abnormality. Ten minutes after infusion of PI (0.1 percent; $n = 3$), granular staining was found primarily in Purkinje dendrites. Labeling was heaviest in lobules I and II of the anterior vermis and lobules IX and X of the posterior vermis, which would be the first segments of the cerebellum to be contacted by substances in the CSF exiting from the fourth ventricle. Neurons around the injection cannula were also labeled, but there was no consistent staining of other brain regions at this time. By 30 minutes after infusion, granular deposits of dye were more prominent in dendrites and were also present in Purkinje somata. At 90 minutes, when the rats' behavior had returned to normal, the somata of Purkinje neurons were labeled heavily while the dendritic staining was lighter. Scattered neurons adjacent to the anterior third ventricle and within the superficial brainstem reticular formation were also la-

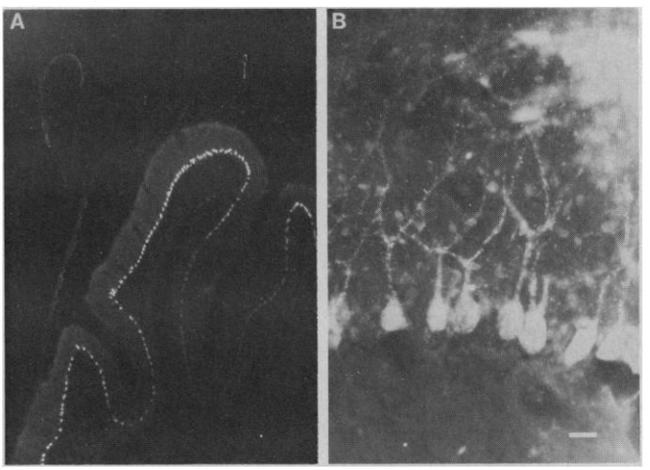

Fig. 1. (A) Low-power view of the anterior cerebellar vermis 1 hour after intracerebroventricular infusion of PI (0.2 percent). Purkinje neurons are labeled brightly. The Purkinje neurons in the depths of a sulcus are labeled less brightly, demonstrating that the cerebellar molecular layer in the sulcus is less exposed to substances injected into the CSF. (B) High-power view of an area of cerebellar vermis that was labeled less heavily. The granularity of the label in the proximal dendrites and somata is more apparent. Purkinje cell axons are not labeled. The coordinates used for placement of the intraventricular cannula were 0.4 mm posterior to bregma, 2.0 mm lateral to the midline, and 3.2 mm deep to the skull (*9*). Scale bar, 25 μm.

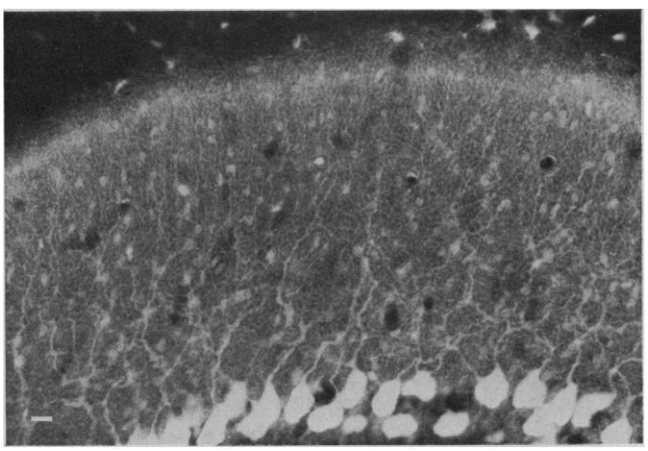

Fig. 2. High-power view of a brightly labeled area of the molecular layer shown in Fig. 1A. Labeling is primarily within the somata and dendrites of Purkinje neurons. Purkinje axons are not labeled. Scale bar, 25 μm.

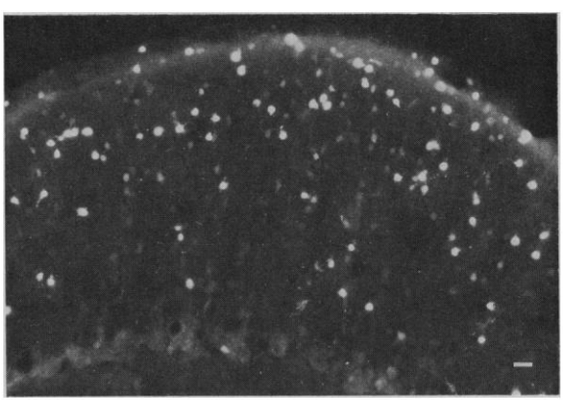

Fig. 3. High-power view of the anterior cerebellar vermis of a rat that received ouabain intracerebroventricularly before the intracerebroventricular infusion of PI (0.2 percent). This rat did not demonstrate the behavioral abnormality that is characteristic of PI infusion. The tissue was prepared exactly like that shown in Fig. 2 and the same area of the anterior vermis was photographed. Purkinje neurons are virtually unlabeled. The globular, highly refractile structures represent PI labeling in round structures in the molecular layer. Rats treated with colchicine demonstrated the same lack of Purkinje neuron labeling but did not contain PI labeling in the round structures. Scale bar, 25 μm.

beled at this time. These histological observations suggest that Purkinje dendrites were transporting the dye through the molecular layer. The period of behavioral abnormality seemed to correspond to the period during which the PI passed through the molecular layer. At later times, rats with heavily labeled Purkinje cell bodies and sparse dendritic labeling behaved normally.

Although the temporal pattern of cerebellar labeling suggested an active, retrograde dendritic transport of PI, it was also possible that the dye was diffusing through the molecular layer and simply staining the Purkinje neurons. To assess this possibility, we studied the effects of pretreatment with ouabain (*10*) and colchicine (*11*), drugs known to block neuronal transport processes (Fig. 2). Rats received intracerebroventricular infusions of PI (0.1 percent) 1 hour after ouabain (10 nmol in 10 μl; $n = 4$) or 14 hours after colchicine (0.4 mol in 10 μl; $n = 4$) had been infused into the same lateral ventricle. Rats treated with either of these compounds did not show the characteristic behavioral abnormalities associated with intracerebroventricular infusion of PI. In addition, these agents virtually eliminated accumulation of PI by Purkinje neurons and their dendrites. Since the dye accumulation and behavioral abnormalities were not reduced when colchicine and PI were infused together, it is unlikely that simple binding of colchicine to PI would explain these observations.

Ouabain blocks active transport by inhibiting Na^+- and K^+-dependent adenosinetriphosphatase (*12*), while chochicine causes dissociation of microtubules (*11*). Inhibition of labeling by ouabain or colchicine suggested that active microtubular transport is necessary for Purkinje neurons to accumulate PI from the CSF. Furthermore, the observation that ouabain and colchicine also prevented the behavioral abnormalities suggests that these, too, are the result of active uptake and neuronal transport of PI.

Propidium iodide causes an orange discoloration of the CSF after intravenous administration (*6*), suggesting that it can cross the blood-CSF barrier. Therefore we examined the behavioral response and neural distribution of PI administered intravenously. Propidium iodide (8 mg/kg) injected into the tail veins of unanesthetized rats ($n = 4$) caused only mild, transient hyperactivity, not tremor. Higher doses caused cardiac arrest. One hour after infusion, the dye was observed in the dendrites and somata of Purkinje neurons in lobules IX and X. Although the concentration of dye was less than had been seen after intraventricular administration, the same pattern of labeling was observed. There was no evidence that PI can pass directly through the blood-brain barrier. An additional three rats were treated with acetazolamide (50 mg/kg, intraperitoneally) to reduce CSF flow (*13*) 4 hours before being intravenously infused with PI. Dye labeling of cerebellar Purkinje neurons in these animals was further reduced. These observations demonstrate that Purkinje neurons can also be labeled by intravascular components that enter the CSF. The absence of behavioral abnormality in these animals can be attributed to the lack of sufficient PI reaching the cerebellar vermis.

Propidium iodide is a polar phenanthridine compound that binds selectively to nucleic acids (*14*). Nucleic acids gain access to the central nervous system by being transported through the choroid plexus to the CSF (*15*), and are incorporated into the nuclear DNA of postmitotic Purkinje neurons (*16*). Therefore it is possible that Purkinje neurons extract PI from the CSF through a physiological mechanism that is designed to supply the Purkinje neurons with nucleic acids. Purkinje neurons can also extract large proteins such as HRP-WGA, lectins (*3*), and immunoglobulins (*4*) from the CSF. The mechanism by which Purkinje neurons accumulate these compounds selectively remains unknown, but may be similar to the mechanism used by Purkinje neurons to pinocytose and degrade parallel fiber synaptic membranes during development (*17*). It is possible that adult Purkinje neurons retain this pinocytic capability and use it to accumulate compounds having access to synapses in the molecular layer.

Shortly after the onset of the postural tremor following intracerebroventricular administration of PI, PI staining was most prominent in the outer molecular layer of the anterior and posterior cerebellar vermis. The return of normal behavior was associated with the accumulation of PI in the somata of the Purkinje neurons. Since this behavioral abnormality has many similarities to the archicerebellar syndrome associated with vermis pathology (*18*), it is tempting to postulate that the PI that gained access to the cerebellar vermis caused a physiological dysfunction. Further studies will be necessary to elucidate the mechanism by which PI causes neuronal disturbance.

Purkinje neurons are affected by many diverse pathological agents (*19*), and it is possible that some agents gain access to Purkinje neurons by being extracted from the CSF. The clinical significance of these findings is further emphasized by the observation that some compounds, including PI, can pass from the vasculature to the CSF and then to the Purkinje neurons. Rigorous support of these possibilities and a full understanding of this newly recognized ability of Purkinje neurons to extract molecules from the CSF must await further study.

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