Cerebellar Purkinje Cell Projection to the Peripheral Vestibular Organ in the Frog

Abstract. *Neurones located 200 to 300 microns from the surface of the auricular lobe of the frog cerebellar cortex, and identified as Purkinje cells, were activated antidromically from the eighth cranial nerve. A parallel anatomical study confirmed the existence of this projection. On the basis of these findings the existence of a cerebello-vestibular efferent system is postulated, the precise significance of which is as yet unclear. However, since Purkinje cells in other species have an inhibitory action on their target cells, the Purkinje efferent system to the vestibular organ may have an action similar to that ascribed to the olivo-cochlear bundle upon the cochlea, that is, to serve as an inhibitory control system.*

An outstanding characteristic of the neural organization of the cerebellum throughout evolution has been the presence of only one efferent system from the cerebellar cortex—the Purkinje cell axons. While most of these axons project directly to the underlying cerebellar nuclei (*1, 2*), anatomists have described many extranuclear projections in different species (*2, 3*). Recent physiological evidence has confirmed the existence of these extranuclear projections (*4*) and, in addition, has shown that Purkinje cells have an inhibitory action on cerebellar and vestibular nuclei (*4*). Furthermore, the axon collaterals of Purkinje cells are inhibitory on cerebellar basket cells (*5*) and directly to Purkinje cells themselves (*6*).

So far, however, the cerebellar projections described have been restricted to systems involved in motor control, no direct relation having been found between cerebellar efferents and primary sensory systems. Our experiments, on the other hand, indicate that, in the frog, auricular Purkinje cells project directly to the peripheral vestibular organ.

The general experimental procedures have been described (*7, 8*). Bullfrogs (*Rana catesbiana*) were anesthesized with pentobarbital sodium (60 mg per kilogram of body weight), and the eighth nerve was stimulated electrically by means of bipolar electrodes located extracranially in its anterior or posterior branches (Fig. 1A). Extracellular and intracellular recordings from Purkinje cells were performed with micropipettes filled with 4*M* NaCl and 3*M* KCl, respectively. The average direct-current resistance of the extracellular recording electrode was 5 megohms; that of the intracellular one was 10 megohms. As in previous experiments (*8*), the threshold current for electrical stimulation of the eighth nerve was measured at the initiation of the experiment and was never increased above 2.5 times threshold.

Electrical activation of the eighth nerve evokes activation of Purkinje cells in the frog auricular lobe via climbing and mossy fibers (*8*). The activation of cat (*9*) and frog Purkinje cells (*10*) by climbing fibers has been characterized by an all-or-none burst of spikes having a very regular pattern of discharge and an almost constant latency. Activation by mossy fibers, on the other hand, produces, in the cat, activation of Purkinje cells which has variable latency (*11*). Similar findings have been reported for the frog cerebellum (*7, 8, 12*). Our report, however, deals almost exclusively with Purkinje cell activation of short latency, which has been ascribed to the antidromic invasion of Purkinje cells (*7, 12*).

Giant extracellular positive-negative action potentials were recorded from the frog auricular lobe after threshold electrical stimulation of the eighth nerve (Fig. 1, B and C). Four criteria were used to identify these action potentials as those generated by Purkinje cells: (i) their antidromic activation from extracerebellar regions (*6, 10, 12, 13*), (ii) their characteristic large extracellular action potentials, the so-called "giant spikes" (*13*), (iii) their orthodromic activation by stimulation of parallel fibers (*7, 10, 14*), and (iv) in the auricular lobe, their depth from the piaglial membrane, that is, 200 to 300 μ from the surface (*8*). Forty-seven cells were studied; their latency is illustrated in Fig. 1K. A large number of Purkinje cells activated at longer latencies were not included since their latencies put them outside the scope of this paper.

There appear to be two groups of action potentials activated by stimulation of the eighth nerve (Fig. 1K). The

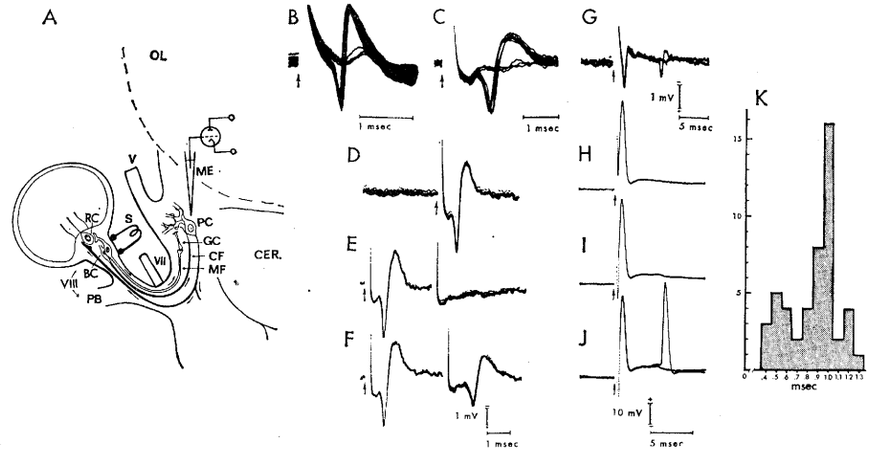

Fig. 1. (A) Diagram of frog brainstem, labyrinth, and experimental arrangement; *BC*, bipolar ganglion cells; *CER*, cerebellum; *CF*, climbing fiber; *GC*, granule cell; *ME*, recording microelectrode; *MF*, mossy fiber; *OL*, optic lobe; *PC*, Purkinje cell; *RC*, receptor cell; *S*, peripheral nerve stimulating electrode; *V*, trigeminal nerve; *VII*, facial nerve; *VIII*, stato-acoustic nerve; *PB*, posterior branch; arrows indicate the direction of impulse conduction. (B–F) Antidromic extracellular action potentials from Purkinje cells in the auricular lobe, recorded at a depth of 250 μ and evoked by stimulation of the eighth nerve. (B and C) All-or-none spike responses at threshold intensity from two different Purkinje cells. (D–F) Purkinje cell action potentials evoked by activation of the eighth nerve at 1.3 times the threshold. (D) Control. (E) Refractory period for antidromic invasion of the Purkinje cell after double shocks with a short interval between stimuli. (F) As in E, but with longer interval between stimuli, showing second Purkinje spike. (G and H) Extracellular and intracellular recording from a Purkinje cell in the auricular lobe at a depth of 300 μ activated antidromically and synaptically by stimulation of the eighth nerve. (G) Extracellular record. (H and I) Intracellular records of the same cell showing the antidromically excited action potential and an excitatory postsynaptic potential. (J) At slightly higher stimulus intensity the Purkinje cell shows also a transsynaptic response. (K) Latency histogram of the early spike responses after stimulation of the eighth nerve. Abscissa, time in milliseconds. Ordinate, number of Purkinje cells recorded. The arrows in B–J indicate stimulus artifact location. Time and voltage calibration as indicated. The polarity is expressed by the positive and negative signs on the amplitude calibrating bar.

first group has its peak at 0.5 msec, whereas the second has a peak at about 1.0 msec. Cell B is an example of a cell having an action potential with a short latency, which is about 0.5 msec; cell C has a latency of approximately 0.9 msec.

All the cells in Fig. 1K could follow repetitive activation ranging from 200 to 400 stimulations per second and demonstrated a refractory period in the range of 3 to 4 msec with a relatively small shift in latency for the second invasion near the refractory period (shift less than 0.5 msec). Figure 1, D to F, illustrates the refractory period of cell C which was 3 msec for the antidromic invasion. If the interval between the two stimuli was increased to 3.2 msec (Fig. 1F), a smaller action potential was recorded (see Fig. 1D) which had a larger initial segment-somadendritic (IS-SD) separation (*6*).

The extracellular antidromic action potentials evoked by stimulation of the eighth nerve have characteristics very similar to those already recorded by Matthews *et al.* (*12*) who demonstrated that, when a pair of stimuli are delivered at short intervals to the cerebellar white matter, there is a delay in the second response of the Purkinje cell. We frequently observed this phenomenon during our experiments (Fig. 1F). Furthermore, there was, in many instances, a large reduction of the amplitude of the second action potential, suggesting that the cell had not completely recovered from the preceding spike (Fig. 1F).

Figure 1, G to J, illustrates extracellular and intracellular recordings from other Purkinje cells after stimulation of the eighth nerve. In Fig. 1G, an activation of the eighth nerve with a stimulus 1.7 times greater than the threshold stimulus evoked an antidromic as well as an orthodromic action potential. In Fig. 1, H to J, a similar cell from the same electrode tract was impaled intracellularly. As the stimulus strength increased from 1.2 to 1.5 times the threshold stimulus, a subthreshold excitatory postsynaptic potential was seen (Fig. 1I). In Fig. 1J an orthodromic activation was obtained with a stimulus strength of 1.8 times the threshold strength. In Fig. 1, I and J, the stimulus artifact interfered with the rising phases of the antidromic action potential. As in previous studies, intracellular impalement of frog Purkinje cells proved very difficult, for which reason both the resting potential (approximately 40 mv) and the action potential were smaller than expected (*7, 12*).

The orthodromic activation of Purkinje cells after stimulation of the eighth nerve may be ascribed to at least three pathways; two originate directly from the vestibular bipolar cells (Fig. 1A), and the third is the vestibulocerebellar projection through the vestibular nuclei. The two direct pathways end as climbing and mossy terminals in the cerebellar cortex (*8*). However, since activation of auricular Purkinje cells by climbing fibers can be recognized by its short latency and characteristic burst activation (*8*), the aforementioned response must be evoked by mossy terminals. It has not been determined which of the two systems of mossy fibers, the direct vestibulo-cerebellar system or the disynaptic system through the vestibular nuclei, is responsible for the activation in Fig. 1, G and J. Since the latency is fairly long, (7.5 and 5.0 msec) however, these action potentials may be evoked by disynaptic vestibulo-cerebellar fibers.

On the basis of these data, we conclude that a number of Purkinje cells from the auricular lobe project directly

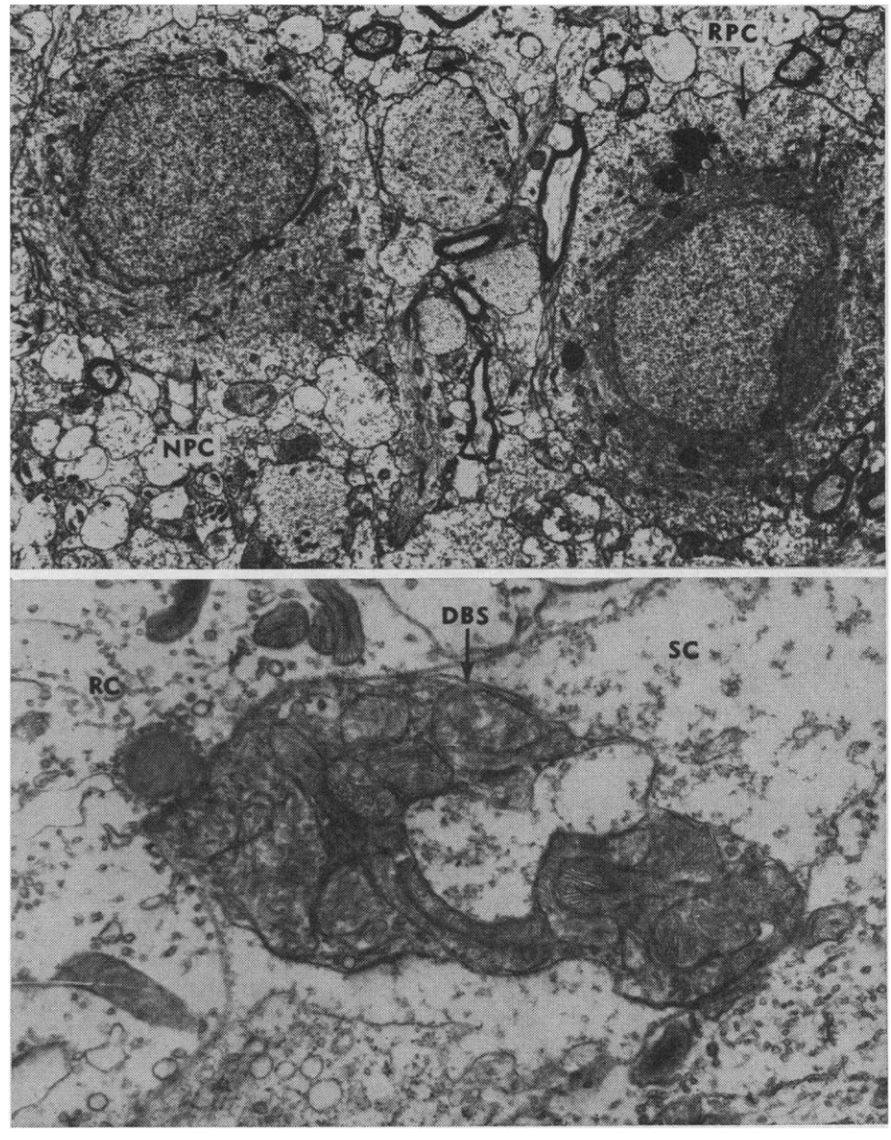

Fig. 2. Electron micrograph of retrograde changes in a Purkinje cell and degenerating synaptic terminal on to vestibular receptor cells. (Top) Retrograde changes in a Purkinje cell of frog auricular lobe 4 days after ipsilateral transection of the stato-acoustic nerve extracranially (*RPC*). Note the dark ring formed around the cell nucleus by the rearrangement of the endoplastic reticulum; note also the normal appearance of the adjacent Purkinje cell (*NPC*) (× 5500). (Bottom) Degenerating synaptic terminal 3 days after ipsilateral removal of cerebellar cortex in the frog (*DSB*). Note the darkness of the terminal and the clustering of the mitochondria. *RC*, vestibular receptor cell; *SC*, supporting cell (× 39000).

to the peripheral vestibular organ, and that there appear to be two groups with different conduction velocities. Moreover, a parallel light- and electron-microscopic study has confirmed the existence of the direct cerebello-vestibular pathway in the bullfrog by showing that section of the eighth nerve extracranially produces degeneration of both climbing and mossy fibers, as well as characteristic retrograde changes in the somas of Purkinje cells (*15*). The latter finding (Fig. 2, top) directly confirms the thesis that Purkinje cells send axons or axon collaterals to the vestibular organ. In addition, if the cerebellar cortex is removed, care being taken not to injure the cerebellar nuclei, one can demonstrate degenerating synaptic boutons in contact with the peripheral vestibular receptor cells (Fig. 2, bottom) (*15*).

The aforementioned results demonstrate the existence of a cerebello-vestibular efferent system in the frog. Previous anatomical and physiological studies have indicated the presence of an efferent system to the peripheral vestibular organ. In the case of the cat, light- (*16*) and electron-microscopical studies (*17*) have revealed efferent terminals on the vestibular receptor cells. Furthermore, direct-current potential changes have been recorded from the surface of the semicircular canals following stimulation of the central nervous system (*18*). In the frog, recent electron-microscopical studies have verified the existence of this efferent system (*19*), which had been suspected on the basis of earlier physiological findings (*20*). The origin of the efferent system was, however, unknown.

Since the cerebellum develops in very close relation with the stato-acoustic system and since a certain component of the vestibular projection to this center is direct in both lower (*21*) and higher vertebrates (*2*), it seems possible that the cerebellum has some direct action upon the peripheral organ. Occasionally we observed direct-current potential changes at the frog vestibular organ following the stimulation of the auricular lobe; however, no clear physiological meaning has so far been attached to this finding.

Although the functional significance of the cerebello-vestibular system has not been ascertained, this system may be inhibitory in nature, as is the olivo-cochlear bundle (*22*), particularly since Purkinje cells inhibit all target cells so far studied (*4*). The demonstration of a direct cerebello-vestibular pathway in the frog implies that, at least in this form, the cerebellum is not involved in motor control exclusively, but has a sensory regulatory role as well.

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11 September 1967

Erythrocyte Transfer RNA: Change during Chick Development

Abstract. *Radioactive aminoacyl transfer RNA's isolated from erythrocytes in the blood of 4-day-old chick embryos and from reticulocytes of adult chickens were analyzed by chromatography on methylated albumin kieselguhr and freon columns. Embryonic and adult methionyl transfer RNA's showed qualitative and quantitative differences in both chromatographic systems. The patterns for arginyl, seryl, and tyrosyl transfer RNA's in the two cell types were similar, while the leucyl transfer RNA patterns suggested a difference.*

Structural modification of transfer RNA (tRNA) may play a regulatory function in cell differentiation and metabolism (*1*). With methylated albumin kieselguhr (MAK) chromatography, Kano-Sueoka and Sueoka (*2*) demonstrated an alteration in leucyl-tRNA after bacteriophage T2 infection of *Escherichia coli*; this finding was confirmed by Waters and Novelli (*3*), using the reversed-phase chromatography developed by Kelmers *et al.* (*4*). Kaneko and Doi (*5*) found a change during sporulation of *Bacillus subtilis* in the elution pattern of valyl-tRNA from MAK columns.

To look for changes in specific tRNA's during development, we used avian immature red cells as a test system and compared the chromatographic profiles of aminoacyl-tRNA's from red cells present in the blood of 4-day-old chick embryos and of reticulocytes of adult chickens. The techniques in this study were MAK (*2*) and freon columns (*6*). Of the five aminoacyl-tRNA's examined, only the methionyl-tRNA gave a strikingly different elution pattern.

Adult chickens (White Leghorn) were made anemic by daily injection (for 5 days) of 15 mg of phenylhydrazine [in 0.1*M* tris(hydroxymethyl)aminomethane (tris) buffer, *p*H 7.2] and were bled on the 6th day. Eggs from the same strain were incubated at 37°C for 4 days, and blood cells were collected by bleeding the embryos. Blood cells from either source were washed twice with 0.145*M* NaCl, $5 \times 10^{-4}M$ KCl, and 0.0015*M* $MgCl_2$, and once with 0.145*M* NaCl, $5 \times 10^{-4}M$ $FeCl_2$, and 0.01*M* phosphate buffer (*p*H 7.4); 0.12 ml of packed embryonic cells was suspended in the latter medium supplemented with glucose (200 mg/ml) with a total volume of 0.5 ml. For adult cells, 0.5 ml of packed cells was similarly incubated in a total volume of 2.0 ml. To cells previously incubated at 38°C for 10 minutes, ^{14}C- or ^{3}H-labeled amino acid (5 to 10 μc) and the 19 remaining nonradioactive amino acids (1 μmole of each) were added. After incubation for an additional 12 minutes, cycloheximide ($10^{-4}M$) was added to inhibit protein synthesis (*7*) and thus prevent the trans-