chemical and kinetic properties and the enzymatic interconversion of the a and b forms of this hepatoma phosphorylase require further study.

KIYOMI SATO
HAROLD P. MORRIS
SIDNEY WEINHOUSE

Fels Research Institute and Department of Biochemistry, Temple University School of Medicine, Philadelphia, Pennsylvania 19140, and *Department of Biochemistry, Howard University, Washington, D.C.*

References and Notes

1. C. M. Goodall, *Int. J. Cancer* **4**, 1 (1969); M. B. Lipsett, *Ann. Int. Med.* **61**, 733 (1964).
2. R. T. Prehn, *Fed. Proc.* **24**, 1018 (1965).
3. L. Lengerova, *Advan. Cancer Res.* **16**, 235 (1972).
4. T. Ono and S. Weinhouse, Eds., *Isozymes and Enzyme Regulation in Cancer* (Gann Monogr. 13, Japanese Foundation for Cancer Research, Tokyo, in press).
5. S. Weinhouse, *Cancer Res.* **32**, 2007 (1972).
6. G. I. Abelev, *Advan. Cancer Res.* **14**, 295 (1971); P. Gold, *Progr. Exp. Tumor Res.* **14**, 43 (1971).
7. W. E. Knox, *Enzyme Patterns in Fetal, Adult and Neoplastic Rat Tissues* (Karger, Basel, 1972).
8. M. M. Appleman, E. G. Krebs, E. H. Fisher, *Biochemistry* **5**, 2101 (1966).
9. F. Schapira, *Pathol. Biol.* **18**, 209 (1970); ——— and C. Gregori, *C. R. Acad. Sci.* **272**, 1167 (1971); T. Matsushima, S. Kawake, M. Shibuya, T. Sugimura, *Biochem. Biophys. Res. Commun.* **30**, 565 (1968).
10. F. A. Farina, R. C. Adelman, C. H. Lo, H. P. Morris, S. Weinhouse, *Cancer Res.* **28**, 1897 (1968); V. R. Potter, *Exploitable Molecular Mechanisms and Neoplasia* (an Annual Symposium on Fundamental Cancer Research) (Williams & Wilkins, Baltimore, 1969); G. Weber, *Naturwissenschaften* **55**, 418 (1968).
11. E. M. Fischer, M. G. Heilmeyer, Jr., R. H. Haschke, in *Current Topics of Cellular Regulation*, B. L. Horecker and E. R. Stadtman, Eds. (Academic Press, New York, 1971), vol. 4, pp. 211–265; H. DeWulf, *The Control of Glycogen Metabolism in the Liver* (Vancer, Louvain, 1971).
12. V. N. Nigam and A. Cantero, *Advan. Cancer Res.* **16**, 1 (1972).
13. E. Sutherland, *Methods Enzymol.* **1**, 215 (1955).
14. C. L. Sevilla and E. H. Fischer, *Biochemistry* **8**, 2161 (1969).
15. L. H. Schliselfeld, C. H. Davis, E. G. Krebs, *ibid.* **9**, 4959 (1970).
16. This work was supported by grants from the National Cancer Institute, CA-12227, CA-10916, and CA-10729; and BC-74M from the American Cancer Society.

25 July 1972; revised 14 September 1972

Synaptic Transmission at Single Glomeruli in the Turtle Cerebellum

Abstract. *We have recorded from the granular layer of the turtle cerebellum extracellular unitary potentials that appear to reflect pre- and postsynaptic events at the synapse between a single swelling of a mossy fiber and the dendritic tips of several granule cells. The presynaptic component is an all-or-none potential. It can be directly activated by spinal stimulation and is unaltered by repetitive activity or by high concentrations of magnesium. The postsynaptic component is a graded potential. It follows the presynaptic component by approximately 1 millisecond and is depressed by repetitive activity and by high concentrations of magnesium. The recording of large potentials produced by the flow of postsynaptic current within a single glomerulus suggests powerful transmission. Electron micrographs demonstrate large cerebellar glomeruli in the turtle and a substantial accumulation of mitochondria in the dendritic tips of granule cells.*

We report here the electrical activity and structure of single glomeruli in the turtle cerebellum. Specimens of *Pseudemys scripta elegans* were sedated with sodium pentobarbital (3 mg/kg) and immobilized with gallamine (1 to 2 mg/kg), both injected intraperitoneally, and were respirated artificially. Tungsten or steel microelectrodes (*1*) having impedances of 1 to 3 megohms (1 khz) were advanced through a slit in the dura. The unconvoluted, layered structure of the turtle cerebellum ensures that the microelectrode records sequentially from the molecular, Purkinje cell, and granular layers. The characteristic "simple" and "complex" spikes of Purkinje cells (*2*) provided one physiological landmark and the abrupt quiescence on entering the fourth ventricle another. This routine method for locating the granular layer was confirmed in 18 cases by histological marking techniques (*1*).

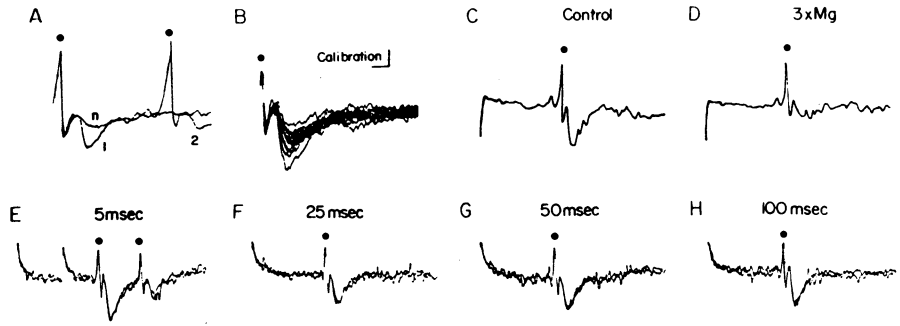
We have recorded a variety of unitary potentials from this granular layer. The most striking potential consisted of an all-or-none, positive-negative biphasic spike followed, after a brief delay (0.8 to 1.6 msec), by a slower negative wave of variable amplitude (Fig. 1). The initial portion is designated the B (biphasic) potential and the subsequent negative deflection, the negative afterwave (NAW). Together they are referred to as a B complex (*3*). We have isolated 34 B complexes in the course of 87 experiments. Although encountered less frequently than other wave shapes, B complexes have some unusual features which make them the subject of this report.

The B complexes shown in Fig. 1A were elicited by a maintained extension of the left hind limb. This produced a train of B potentials, each of which was followed by a NAW. The two superimposed traces (Fig. 1A) show only three discharges from the train; the B potentials are marked by dots and the first, second, and *n*th NAW's are labeled. The amplitude of the NAW decreased after the first discharge, whereas the B potential was unchanged. Another unit (Fig. 1B) responded with a train of B complexes when the right hind limb was touched. The superimposed traces, triggered on the rising phase of the B potentials, show each NAW in the train. The amplitude of the NAW declined progressively, the first in the sequence being the largest and the last the smallest.

Responses to electrical stimulation of the spinal cord allowed a more controlled investigation of the depression of the NAW produced by prior activity. The dura overlying the spinal cord was exposed by boring a hole through the carapace and vertebrae. After slitting the dura, we placed a monopolar electrode over the cord in contact with the cerebrospinal fluid. Shocks of 1 to 10 volts (0.1-msec duration) activated many of the units recorded in the granular layer. The B complexes which were studied discharged once at a fixed latency in response to a single shock. The time course of the depression of the NAW was studied by observing the amplitude of the second of the two NAW's elicited by paired shocks delivered to the spinal cord. The depression was maximum for intershock intervals of 5 to 10 msec (Fig. 1E). The NAW progressively increased with longer intervals (Fig. 1, F to H). Complete recovery required up to 1 second.

The all-or-none nature of the B potential indicates that it is a single action potential. The delay between the B potential and the NAW, as well as the graded nature of the latter, suggests that the NAW represents current at postsynaptic structures. A waveform similar to the B complex has been observed with extracellular microelectrode recordings at the giant synapse of *Loligo*, the neuromuscular junction of the rat and crayfish, and in the auditory system (*4, 5*). In all these cases an

Fig. 1. Microelectrode recordings of B complexes in the granular layer of the cerebellum. (A) Three discharges in a train elicited by natural stimulation. The first, second, and *n*th NAW's in the sequence are labeled in the two superimposed traces. Sweep triggered on rising phase of first and *n*th B-potential portion of the B complex. (B) Entire train of B complexes elicited by natural stimulation, each trace triggered on the rising phase of the B potential. The NAW's of successive B complexes became progressively smaller in amplitude. (C and D) These B complexes were elicited by spinal cord stimulation while the ventricular system was perfused with artificial cerebrospinal fluid having normal ionic concentrations (C) and an elevated concentration of magnesium (D). The B potential is not appreciably affected whereas the NAW is essentially abolished by high magnesium. (E to H) A unit directly activated by shocks delivered to spinal cord. Pair of shocks separated by the designated interval elicits two successive B complexes. (E) Two shock artifacts and two B complexes are shown. (F to H) Only the second of the two complexes is shown. The NAW recovers as the interval increases. In each case there are two superimposed traces to show the fixed latency of the B potential. Positivity upward in all traces. Dots mark B potentials. Calibrations: (A) 1 msec, 100 μv; (B) 1 msec, 50 μv; (C and D) 2 msec, 100 μv; (E to H) 2 msec, 50 μv.



individual presynaptic action potential appeared as a biphasic, positive-negative spike and the excitatory postsynaptic current as a negative wave.

The postsynaptic origin of the NAW was confirmed in three experiments in which the fourth ventricle was perfused with artificial cerebrospinal fluid (CSF) high in magnesium. The technique for perfusion and the composition of artificial CSF were the same as used by Heisey (*6*). Figure 1C shows a B complex recorded while the ventricle was perfused with artificial CSF of normal composition. When the mag-

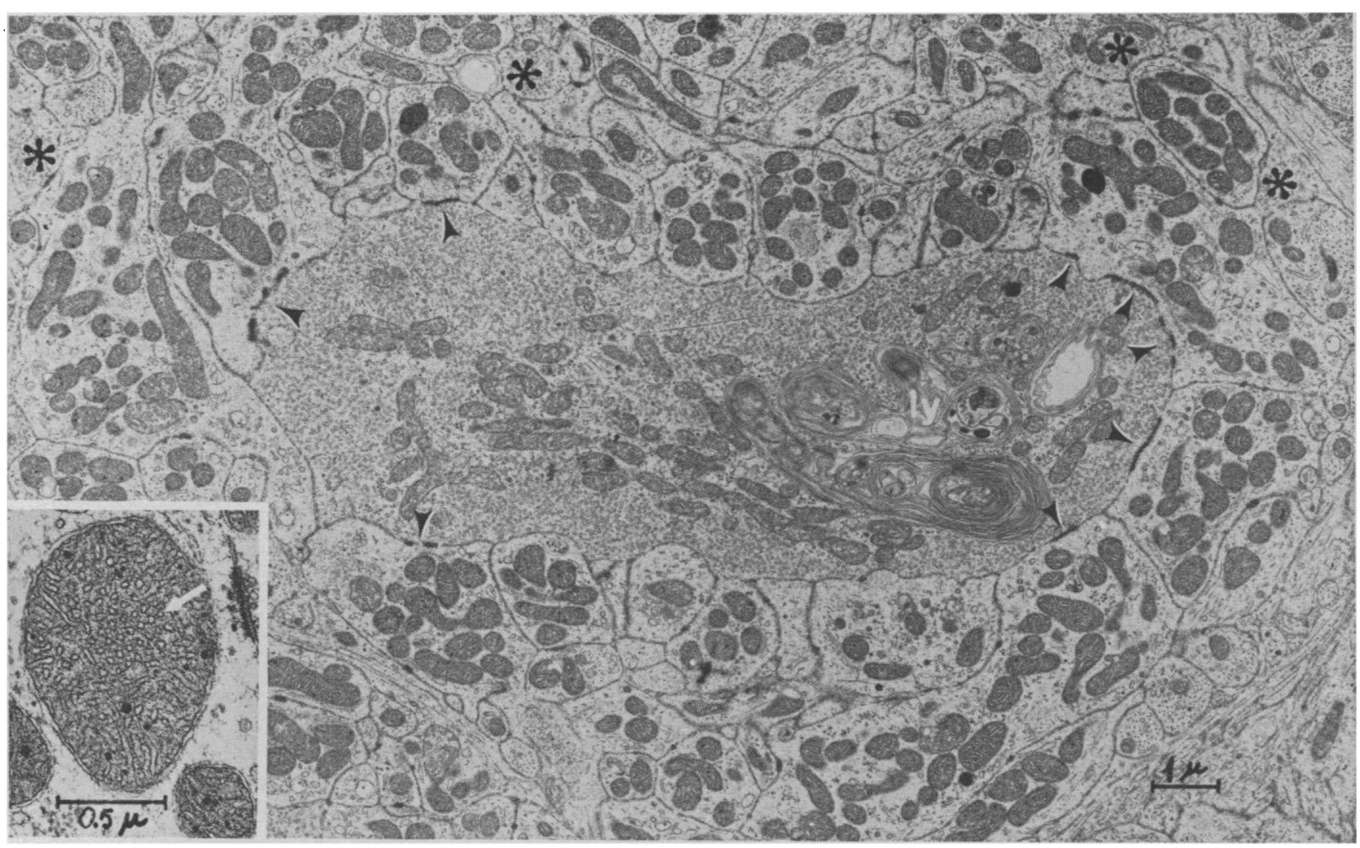


Fig. 2. Electron micrograph showing a glomerulus in the turtle cerebellar granular layer. The central element, containing numerous synaptic vesicles and mitochondria, as well as some cytolysosomes (*ly*), is a portion of the mossy fiber swelling. This has been cut obliquely; therefore, the parent fiber is not included in the picture. The mitochondria-filled profiles are the enlarged terminals of the granule cell dendrites. The mossy fiber swelling is contacted in this plane by 22 dendritic terminals. Other dendritic profiles form a second, more peripheral layer. The arrows point to synaptic junctions. The asterisks mark swellings of the Golgi axonal plexus. The ovoidal glomerulus in this micrograph measures 22 and 12 μm along its major and minor axes, respectively. The diameters of the mossy fiber swelling alone are 15 and 5 μm. The inset shows mitochondria in a dendritic enlargement. Note the numerous cristae, some of which appear rhomboid in cross section and are arranged in parallel rows (arrow). The matrix dense granules are unlabeled. The animals were perfused with buffered formaldehyde-glutaraldehyde. Blocks from the cerebellum were postfixed in osmium tetroxide, dehydrated in methanol and propylene oxide, and embedded in Taab resin–Epon mixture. The ultrathin sections were stained with uranyl acetate and lead. Measurements were repeated in Golgi preparations. The shrinkage taking place in the preparation of this material has not been corrected for in these measurements.

nesium concentration was tripled, the NAW was decreased while the B potential was unaltered (Fig. 1D). It is known that high concentrations of magnesium ions can depress synaptic transmission without affecting the conduction of action potentials (*4, 7*).

Responses of some complexes to electrical stimulation of the spinal cord indicate that they are directly activated without an intervening synapse. The B potentials of these units have fixed latencies (variation less than 0.1 msec) to spinal stimulation, as exemplified by the superimposed traces in Fig. 1, E to H. They also respond with two discharges to a pair of spinal shocks separated by intervals as brief as 1.5 to 3 msec. The fixed latency and ability to follow pairs of shocks strongly suggest that the pathways for these units from the spinal cord to the cerebellum are direct. The only known direct pathways from the caudal spinal cord are the dorsal and ventral spinocerebellar tracts that terminate as mossy fibers in the granular layer. Both tracts have been identified in the turtle (*8*).

We conclude that B complexes represent electrical events at cerebellar glomeruli, the complex synaptic fields where mossy fibers synapse with granule cells. The B potentials represent impulses in synaptic swellings of mossy fibers and the NAW's represent synaptic current in dendrites of granule cells (and perhaps also in dendrites of Golgi cells). A glomerulus is nested in between groups of granule cell bodies, or it may be adjacent to a few other glomeruli involving mossy fibers of different origin. An individual B complex should represent events at a single glomerulus, since we have never recorded simultaneously more than one B complex. If simultaneous recording from adjacent glomeruli were possible under our experimental conditions, we would have been able to recognize it since many mossy fibers were spontaneously active. Furthermore, the NAW component of a B complex was lost when the micromanipulator was advanced by 10 μm or less, whereas individual mossy fibers engage in glomeruli that are rarely spaced less than, and typically much more than, 20 μm apart.

A negative field potential that has been attributed to excitatory synaptic current in a population of granule cells has been observed in several species (*9*); but, recordings from single cerebellar glomeruli have never been reported. Our ability to record these potentials is probably due to the excellent mechanical stability of our preparations in combination with the large dimensions of cerebellar glomeruli. It may also be related to certain synaptic specializations that we have noted in electron micrographs of the turtle cerebellum.

Mossy fibers form en passant (Fig. 2) and terminal swellings 10 to 60 μm long and 3 to 7 μm in diameter. Dendrites of granule cells are 1 μm thick and contact the mossy fiber swellings mainly at their tips, where they form an enlarged ending 2 to 3.5 μm thick and 3 to 8 μm long. These end bulbs are filled with mitochondria and usually form large synaptic junctions or groups of smaller synaptic patches (arrows in Fig. 2). The entire synaptic structure, the glomerulus, has a minor axis ranging from 7 to 20 μm and a major axis ranging from 10 to 60 μm, thus providing a suitable target for extracellular electrodes.

The great density of presynaptic vesicles, the number and extent of synaptic junctions, and the crowding of mitochondria in the dendritic tips are features of this synapse that suggest powerful transmission. This abundance of mitochondria is unusual for synapses in the central nervous system. It has been suggested that a similar accumulation of mitochondria in sensory receptors is related to the energy requirements associated with a large ionic load for the sodium pump (*10*). The mitochondria in the dendritic end bulbs differ from those in mossy fiber swellings by the presence of numerous cristae and matrix dense granules (Fig. 2, inset). Cytochemical investigations might distinguish the enzymatic activities in these dendritic mitochondria, which are probably different from those in the parent cell body and those in the presynaptic terminals (*11*).

While both the electrical and the anatomical data suggest that synaptic transmission from mossy fibers to granule cells is powerful, they provide no direct estimate of the magnitude of the postsynaptic potential. The absence of postsynaptic action potentials, which has been a consistent observation, may indicate that the convergence of two or more inputs is required to fire granule cells. However, we favor the interpretation that action potentials arise in the extremely thin (0.2 μm) initial segment of granule cells and fail to invade the soma and dendrites. Intracellular recordings from granule cells will be required to resolve this important issue.

The turtle seems ideal for the identification of unitary potentials generated at single cerebellar glomeruli. Our study may facilitate the recognition of glomerular potentials in the cat, the animal of choice of most cerebellar neurophysiologists, although cerebellar glomeruli in the cat are somewhat smaller (10 by 20 μm).

J. V. Walsh*
J. C. Houk
Department of Physiology, Harvard Medical School, Boston, Massachusetts 02115

R. L. Atluri
E. Mugnaini
Department of Biobehavorial Sciences, University of Connecticut, Storrs

References and Notes

1. D. H. Hubel, *Science* **125**, 549 (1957); E. J. Furshpan and T. Furukawa, *J. Neurophysiol.* **25**, 732 (1962).
2. R. Granit and C. G. Phillips, *J. Physiol. London* **133**, 520 (1956); W. T. Thach, *J. Neurophysiol.* **30**, 675 (1967).
3. This terminology modifies that suggested when these potentials were first reported [J. V. Walsh and J. C. Houk, *Fed. Proc.* **30**, 663 (1971)].
4. A. Takeuchi and N. Takeuchi, *J. Gen. Physiol.* **45**, 1181 (1962).
5. J. I. Hubbard and R. F. Schmidt, *J. Physiol. London* **166**, 145 (1963); J. Dudel and S. W. Kuffler, *ibid.* **155**, 514 (1961); R. R. Pfeiffer, *Science* **154**, 667 (1966); R. Y.-S. Li and J. J. Guinan, *Mass. Inst. Technol. Quart. Progr. Rep. No. 100* (1971), p. 227.
6. S. R. Heisey, *Amer. J. Physiol.* **219**, 1564 (1970); *Fed. Proc.* **27**, 287 (1968).
7. J. del Castillo and L. Engbaek, *J. Physiol. London* **124**, 370 (1954).
8. O. Larsell, *J. Comp. Neurol.* **56**, 299 (1932).
9. J. C. Eccles, H. Taborikova, N. Tsukahara, *Brain Res.* **17**, 87 (1970); R. Llinas and D. E. Hillman, in *Neurobiology of Cerebellar Evolution and Development*, R. Llinas, Ed. (American Medical Assoc., Chicago, 1969), p. 43; J. C. Eccles, M. Ito, J. Szentagothai, *The Cerebellum as a Neuronal Machine* (Springer-Verlag, New York, 1967), p. 130.
10. J. C. Houk, *Ann. N.Y. Acad. Sci.* **156**, 901 (1969).
11. F. Hajos and S. Kerpel-Fronius, *Exp. Brain Res.* **8**, 66 (19 *J. Cell Biol.* **51**, 216 (1971).
12. We thank Drs. E. Henneman and N. Kiang for commenting on the manuscript. Technical assistance was provided by J. Dunham and W. Keezer. Supported by NIH grants NS08762 and NS09904 and RSA71-17 from the Connecticut Research Commission. J.V.W. was a postdoctoral fellow of the NIH.
* Present address: National Institute of Neurological Diseases and Stroke, Building 36, Bethesda, Maryland 20014.

26 July 1972; revised 5 September 1972