CEREBELLAR ALTERATIONS IN THE WEAVER MOUSE

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ABSTRACT

The fine structure of the cerebellum of weaver mouse was examined and the paucity of granule cells and their axons, the parallel fibers, was confirmed. Unexpectedly, however, the dendritic spines of the Purkinje cells which, in normal animals, are the postsynaptic mates of the parallel fibers, were present. Furthermore, their essential morphology and their staining reactions were indistinguishable from those of the Purkinje cell dendritic spines in normal animals. Possible mechanisms of development are discussed.

INTRODUCTION

The elucidation of synaptic morphology has been a major contribution of the electron microscope (2, 5, 8, 20, 24, 28, 32). By means of this tool, a great variety of synapses have been examined and a great many more are presently under investigation.

The question of synaptic development, too, has been the subject of a number of publications (1, 4, 7, 10, 12, 15, 24, 25, 39). Various models of synaptic development have been offered, most of which differ from one another in the details of the sequence of appearance of the morphological specializations of the pre- and postsynaptic elements.

In all of these reports, it has been tacitly assumed that contact between the two neuronal elements was needed before any specialization occurred. This is certainly a sensible assumption which is confirmed by all the published material concerning synaptogenesis and, indeed, is probably true of other developing systems as well. Quite recently, however, we have made an observation which may be interpreted in a way which challenges this assumption. It was found that in the murine mutant "weaver", specialized Purkinje cell dendritic spines are present without, in most cases, any presynaptic element. A descrip-

tion of these findings forms the contents of the present report.

MATERIALS AND METHODS

Weaver mice and their normal littermates were obtained from the Jackson Laboratory, Bar Harbor, Maine. Mice between the ages of 24 days and 4 mo were examined. The mice were killed by decapitation, the skull was opened, and the cerebellum was immersed in 5% glutaraldehyde in 1/15 M phosphate buffer, pH 7.4. The tissue was cut into thin slices and stored in glutaraldehyde for about 30 min. After fixation, the tissue was processed according to four different methods.

I. Conventional Electron Microscope Preparation

The tissue was postfixed in Dalton's chrome osmium, pH 7.4, dehydrated in an ascending series of alcohols, and embedded in Luft's Epon after two changes of propylene oxide.

II. Uranyl Acetate Impregnation

The procedure was identical to method I above, except for the inclusion of 1% uranyl acetate in the 70% alcohol change during dehydration (27).
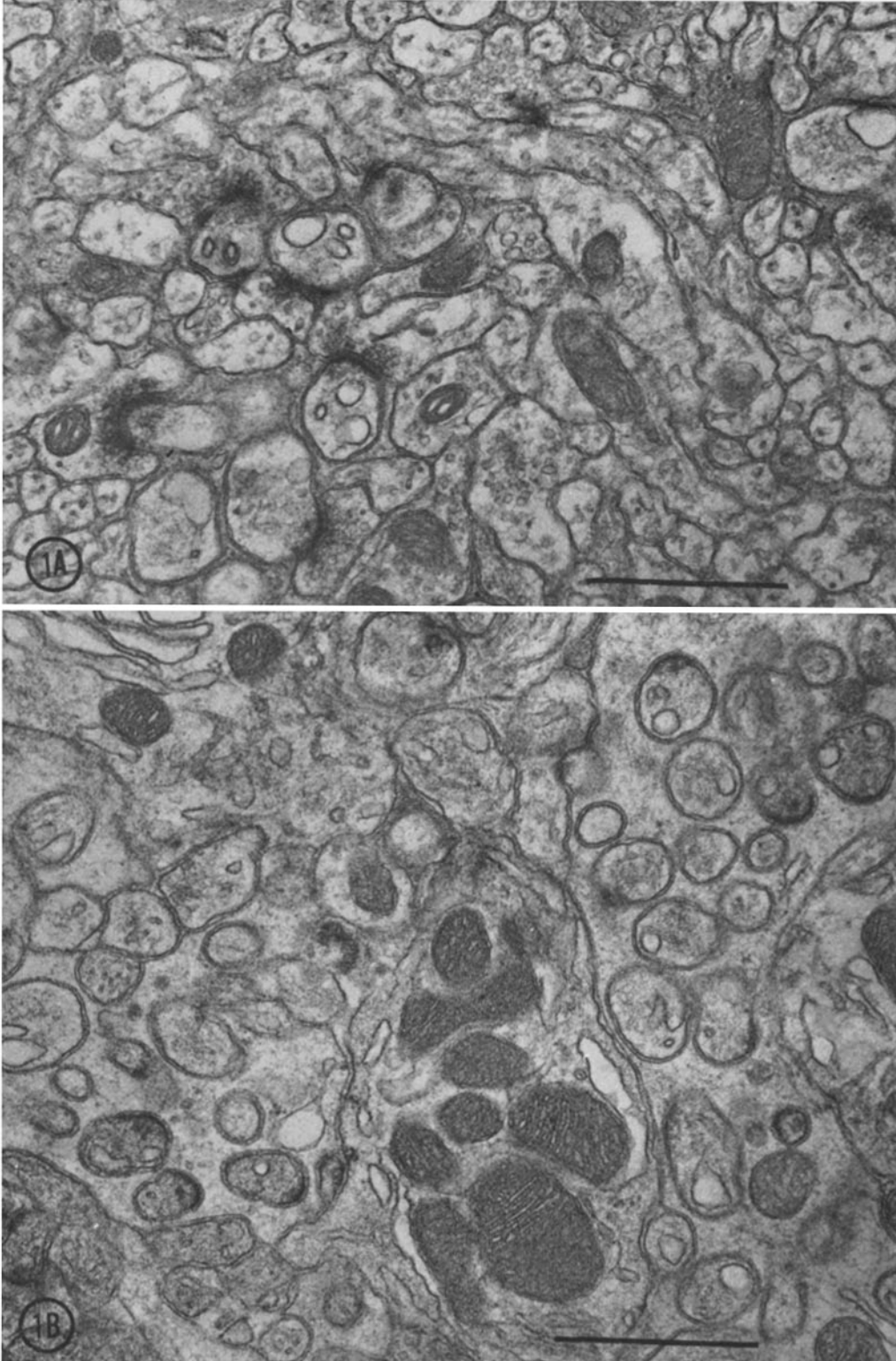
FIGURE 1A The molecular layer of the cerebellum in a normal mouse. Several synapses between Purkinje cell dendritic spines and parallel fibers are visible as well as cross-sections of numerous, small caliber, parallel fibers. × 30,000.

FIGURE 1B The cerebellar cortex of a weaver mouse. Many unattached Purkinje cell dendritic spines are visible within a matrix of astrocytic cytoplasm. Note the paucity of small caliber axons. × 30,000.

III. Ethanolic Phosphotungstic Acid Impregnation

After glutaraldehyde fixation, the tissue was dehydrated to 95% ethanol without osmication. Then, as described by Bloom and Aghajanian (3), the blocks were impregnated for 1 h in 1% phosphotungstic acid in ethanol. The tissue was then immersed in two changes of propylene oxide and embedded in Luft's Epon.

IV. Bismuth Iodide Impregnation

The method used was essentially that of Pfenninger (30). After glutaraldehyde fixation, the tissue was washed overnight in 0.2 M phosphate buffer containing 6.8% sucrose, pH 7.4. The next day, the pH was lowered by immersing in acetate buffer pH 5.0, and then at pH 3.5. After 15 min in each buffer, the tissue was stored overnight in bismuth iodide prepared according to Pfenninger (30). After impregnation, the tissue was washed in 0.1 M phosphate buffer, pH 7.4 with 6.8% sucrose, and then fixed in 2% OsO₄ in the same buffer. After osmication, the tissue was dehydrated and embedded as in method I.

Thin sections were cut and, except for the tissue prepared by method III, were stained in uranyl and lead acetate. The sections were then examined in a Siemens 1A electron microscope.

RESULTS

In the control littermates, the fine structure of the cerebellar molecular layer appeared entirely normal. The details of the anatomy of this portion of the cerebellum and its development have been well described by others (6, 18, 22, 24). The molecular layer consists of a great number of compactly-arranged cell processes, most of which are either parallel fibers or Purkinje cell dendrites and their attached dendritic spines (Fig. 1A). Astrocytic processes are not particularly prominent at low magnification but they invest many of the synapses and other neuronal processes. At higher magnification, the details of the synapses between the synaptic terminals of the parallel fibers and the dendritic spines of the Purkinje cells become clear (Fig. 2A). The presynaptic elements are characterized by the presence of numerous spherical synaptic vesicles. The postsynaptic element is easily recognized by the presence of a layer of electron-opaque material at the cytoplasmic surface of the plasma membrane where it faces the presynaptic element. Between the two elements, the synaptic cleft is wider (ca. 200 Å) than other, nearby extracellular spaces and contains a characteristic electron-opaque material. Usually, the entire synaptic complex is invested by a closely apposed astrocytic process.

The cytoarchitecture of the weaver cerebellum is apparently completely disorganized. Not only is there a severe paucity of granule cells but other elements, including the Purkinje cells, have lost their characteristic orderly alignment. Thus, no clear distinction between the various cortical layers could be made. Nevertheless, the preservation of the remaining cells and processes appeared good (Fig. 1B). No evidence of necrosis or phagocytic activity could be discerned.

The great majority of the neuronal processes found in the cerebellar cortex of the weaver mouse were derived from Purkinje cells. The virtual absence of the small caliber parallel fibers was particularly striking. Nevertheless, the extracellular spaces were not noticeably widened since astrocytic cytoplasm filled the spaces between the remaining neuronal processes. The astrocytic processes were not particularly rich in glial fibers, glycogen granules, or other indicators of reactive changes. As might be expected, full synaptic complexes were rare. On the other hand, dendritic spines of Purkinje cells, often complete with postmembranous densities, were quite common. Dendritic spines could be seen studding almost the

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Figure 2A A synapse between a Purkinje cell dendritic spine and a parallel fiber in a normal mouse. × 112,000.

Figure 2B Unattached dendritic spines in the cerebellar cortex of a weaver mouse. Note the postmembranous densities and cleft material. The extracellular space abutting the region of the postmembranous density is wider than other nearby extracellular spaces. × 112,000.

Figure 3A A synapse between a Purkinje cell dendritic spine and a parallel fiber in a normal mouse stained with ethanolic phosphotungstic acid. × 112,000.

Figure 3B Two unattached dendritic spines in a weaver mouse stained with ethanolic phosphotungstic acid. Both the postmembranous and cleft material take up the stain. × 112,000.
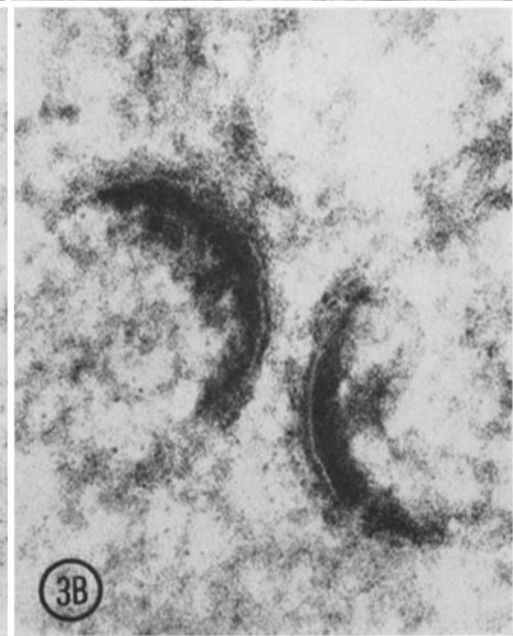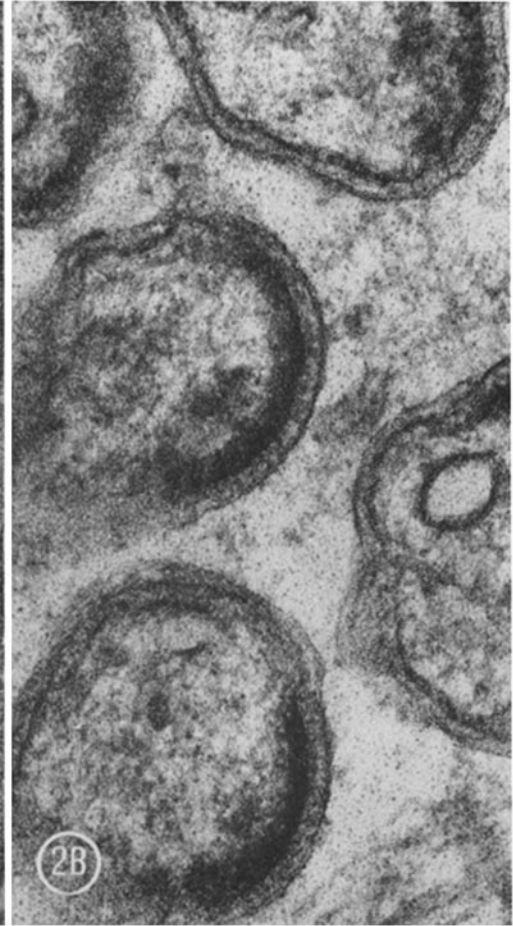
entire surface of even the larger dendritic trunks. As indicated above, they were usually unassociated with any presynaptic element. Instead, they were almost completely surrounded by a matrix of astrocytic cytoplasm. When examined at higher magnification the fundamental fine structure was identical to that of the dendritic spines of the normal cerebella (Fig. 28). Again, a layer of dense material was found at the cytoplasmic surface of a portion of the plasma membrane and, at that surface, the extracellular space intervening between the dendritic spines and the surrounding astrocyte was widened (ca. 200 Å) and contained a layer of electron-opaque material. The only obvious difference between the dendritic spines in weaver and those in normal mice was that in
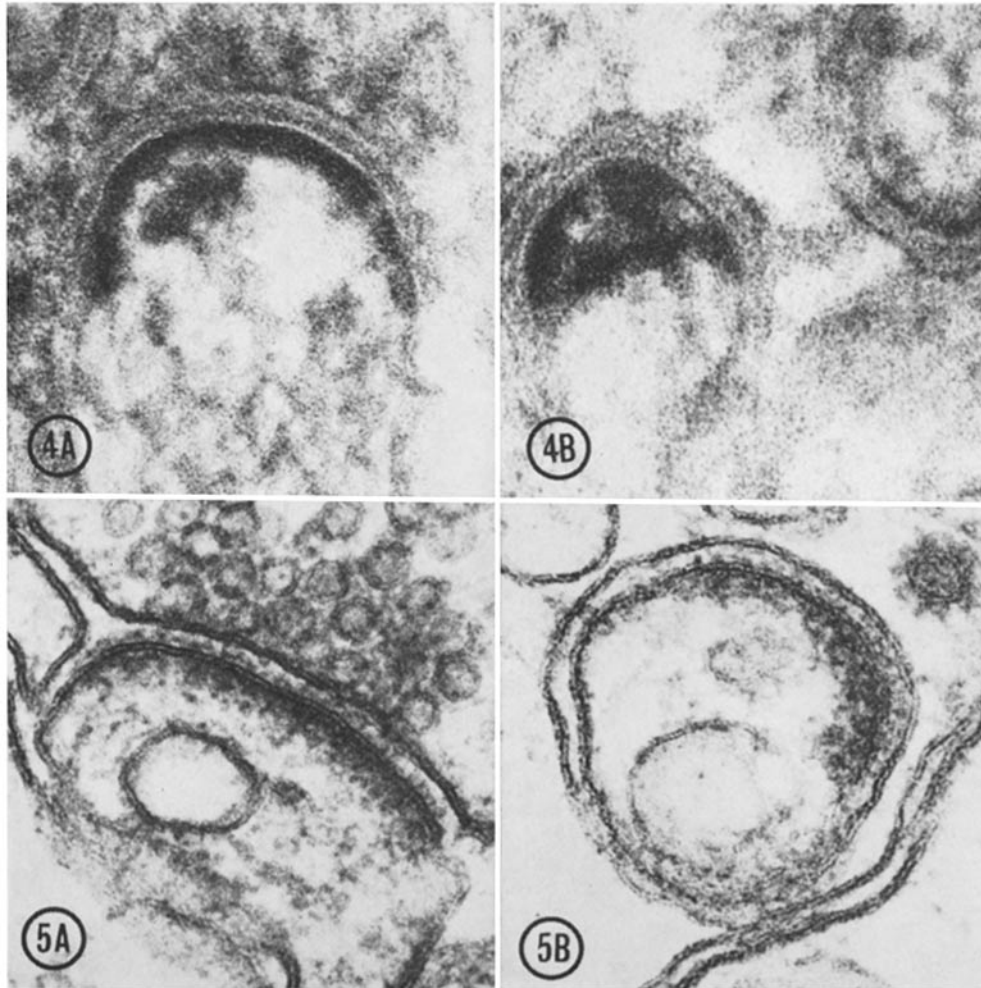

FIGURE 4A A synapse between a Purkinje cell dendritic spine and a parallel fiber in a normal mouse impregnated with bismuth iodide and then stained in section with uranyl and lead salts. × 144,000.

FIGURE 4B Two unattached dendritic spines in a weaver cerebellum treated as described in Fig. 4A. × 144,000.

FIGURE 5A A synapse between a Purkinje cell dendritic spine and a parallel fiber in a normal mouse stained en bloc with uranyl acetate. × 112,000.

FIGURE 5B An unattached dendritic spine in a weaver cerebellum treated as in Fig. 5A. × 112,000.

weaver there was a moderate decrease in the average diameter of the spine and a concomitant decrease in the length of the postmembranous density.

These differences between the normal and weaver cerebella were observed at all the ages examined. The oldest of these was 4 mo, and even at this relatively late stage the unattached dendritic spines of the Purkinje cells, decorated with postmembranous densities, appeared intact (Fig. 6).

When subjected to the special impregnation techniques, no difference could be found between the dendritic spines in the weaver cerebella and those within normal cerebella. Ethanolic phosphotungstic acid treatment of normal mouse cerebella showed a selective staining of the synaptic complex in which, when sectioned at a favorable angle, the presynaptic element was characterized by discontinuous accumulations of electron-opaque material facing the synaptic cleft (Fig. 3A). Material within the cleft also took up the stain. The postsynaptic dendritic spines showed a continuous electron-opaque band facing the synaptic cleft. In weaver cerebella, an identical staining

pattern was observed within the dendritic spine and in the extracellular space directly apposing the stained portion of the spine (Fig. 3B). No characteristic discontinuous electron-opaque accumulations were observed.

The results of bismuth iodide impregnation were essentially similar to those of the ethanolic phosphotungstic acid technique with regard to the cleft material and to the dense deposits within the synaptic elements (Figs. 4A and 4B). In addition, however, a faint outlining of the synaptic vesicles and of all the plasma membranes could be vaguely discerned. In the weaver mouse, the staining pattern of the postsynaptic element and its apposing extracellular space was indistinguishable from that of the normal mouse.

Finally, the use of uranyl acetate, in contrast to the other two special techniques, resulted in particularly well delineated unit membrane structure at both the plasma membrane and the limiting membranes of the synaptic vesicles (Fig. 5A). Dendritic spines within the weaver cerebella reacted identically to those of the normal cerebellum (Fig. 5B).
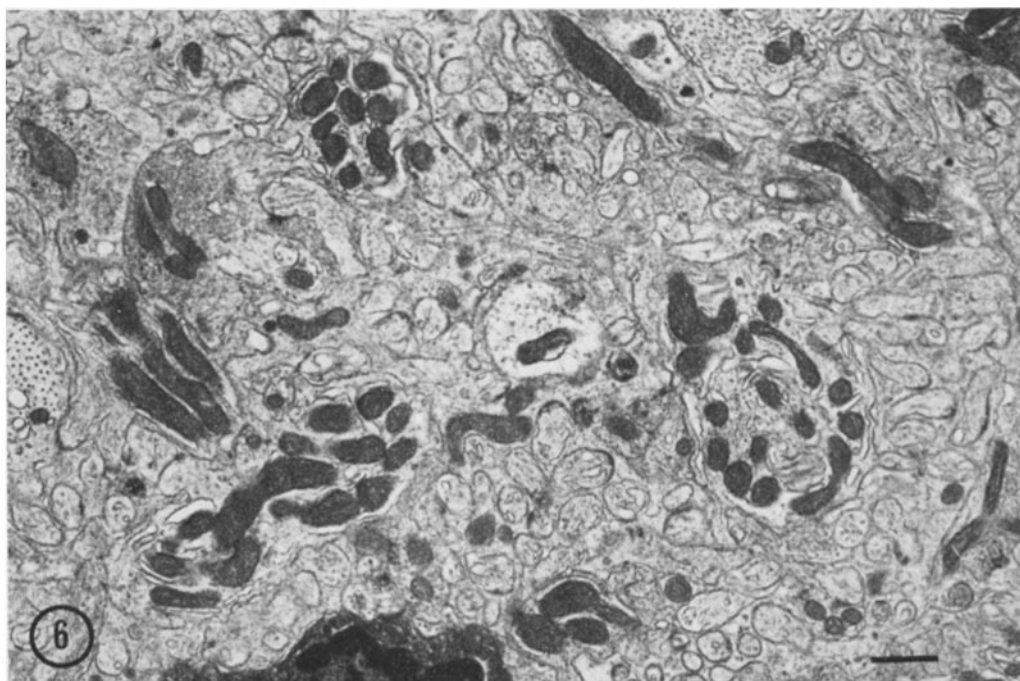

FIGURE 6   A low magnification micrograph of the cerebellar cortex of a 4-mo-old weaver mouse. Numerous unattached dendritic spines are present in the apparent absence of parallel fibers. × 9000.

DISCUSSION

The results of the present study seem clear. As indicated by the previous light microscope studies (35), the weaver cerebellum is drastically deficient in granule cells and their axons, the parallel fibers, and the cerebellar cytoarchitecture is severely distorted. The synapses normally formed by the parallel fibers are, of course, not present but, surprisingly, their most prominent mates, namely the dendritic spines, appear intact, at least in their essential features, by both standard morphological techniques and specialized methods designed to selectively impregnate this structure.

The first explanation of these results which occurred to us is that the granule cells had formed synapses early in development and that these later degenerated, leaving behind the unattached dendritic spines. One might expect that, in such cases, the postsynaptic elements, too, would degenerate (see reference 12). Indeed, experimental studies have demonstrated the degeneration of postsynaptic elements after the destruction of the presynaptic structures in the cerebellum (23) and in other areas of the central nervous system such as the lateral geniculate body (21) in addition to other regions (31).

On the other hand, the persistence of postsynaptic elements, in limited areas at least, after destruction of their presynaptic mates has also been demonstrated (12). Such a process was shown by Herndon (11, 12) who administered thiophen to adult rats and, to a limited extent at least, by Mouren-Mathieu and Colonnier (23) who transected the parallel fibers of adult cats. Similar results have also been obtained in areas of the central nervous system outside the cerebellum (9, 29, 37, 38).

In all of these studies, however, the lesion was induced in adult animals at a time when synapses were definitely present, and, moreover, areas of necrosis and degeneration were obvious in these studies, indicating the recent presence of a severe destructive process. Neither of these two facts is clear in the present study. Sidman et al. (35) have reported that granule cell destruction occurs in weaver "prior to their migration inward to form the granule layer" during the 2nd wk and that symptoms are observable as early as 8 days. According to Larramendi (17, 19), this is before most of these synapses are formed. Thus, it is not at all clear that most Purkinje cell-granule cell synapses were ever formed in the weaver mouse.

We are left then with the unexpected possibility that the unattached Purkinje cell dendritic spines formed independently in the weaver cerebellum and achieved their apparently specialized status without the direct influence of a presynaptic element (12). After some reflection, this possibility took on more credibility. Similar interpretations may be offered in two other cases where Purkinje cell dendritic spines remain apparently intact after granule cell destruction. When feline panleukopenia virus is administered to 1-day-old ferrets (13), or when cycasin is injected into 1-day-old mice (14), the fine structural findings are indistinguishable from those of the present study. In these two studies, also, the possibility of an independent development of Purkinje cell dendritic spines could not be ruled out (12). Finally, a recent report has suggested that, even in apparently normal animals, unattached dendritic structures, indistinguishable from postsynaptic elements, may be found in the olfactory bulb (26).

On the other hand, in another murine mutant "staggerer" (33–36, 40), it has been observed that although the granule cells last substantially longer than in weaver and reach the internal granule cell layer (35), the Purkinje cells do not form unattached dendritic spines (Hirano, unpublished). A recent report suggests that, in staggerer, parallel fibers do not form synapses with Purkinje cells at any time during development (16). Thus if, as we have suggested, Purkinje cell dendritic spines might differentiate in the absence of a presynaptic element, why are they not seen in staggerer? This question cannot, at present, be answered although the possibility that the staggerer mutation affects the Purkinje cell's ability to fully differentiate must be considered.

The only way to test the possibility of an independent formation of the Purkinje cell dendritic spines in the weaver is to explore the synaptology of the neonatal weaver mouse and compare it to that of their normal littermates. Such an investigation is currently underway in our laboratory.

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