Exercise During Development Induces an Increase in Purkinje Cell Dendritic Tree Size

Abstract. *Mice allowed to exercise during the late postnatal period had Purkinje cells with larger dendritic trees and greater numbers of spines than littermates whose physical activity was severely restricted. These changes in Purkinje cells were accompanied by a selective reduction in the thickness of the cerebellar molecular layer. The data provide evidence for cerebellar plasticity during late development and demonstrate that physical activity can modify the development of Purkinje cell dendrites.*

A fundamental challenge of developmental neurobiology is to elucidate the relative roles of invariant (genetic) and variable (environmental) factors in the control of neuronal development (*1*). The importance of environmental stimulation in the development of certain types of neurons has been repeatedly demonstrated. Thus, the effects of functional deprivation (*2, 3*) and stimulation (*4, 5*) on dendritic development have been demonstrated in sensory neurons of the visual system, including the cerebral cortex. On the other hand, the role of function in the development of the motor systems still needs to be established (*6*). To this end, and because the mechanism by which function alters the structural development of the nervous system is obscure, the analysis of simpler brain regions, such as the cerebellum, is needed.

Accordingly, since we had previously studied the normal and abnormal development of the cerebellar Purkinje cell dendrites and had determined that the Purkinje cell is suitable for morphometric analysis (*7, 8*), we investigated the effects of physical activity on the late postnatal development of the Purkinje cell dendritic tree. Quantitation of total branch length and numbers of spines of Purkinje cells was used to determine whether there were alterations in the Purkinje cell dendritic tree after physical activity or inactivity. This study is one of two investigations reported simultaneously in preliminary form (*9*), which appear to be the first demonstrations of cerebellar plasticity during development.

Eleven $B6D2F_1$ mice of both sexes (*10*), from two litters raised in our laboratory, were studied. Littermates were weaned at 18 days of age and separated into two experimental groups until they were killed at 35 days of age. Physically active littermates were raised in a large cage (*11*) containing a variety of exercise apparatuses that allowed free running, climbing, and other movements. They were observed for a period of 2 hours daily and were seen to climb a ladder for water, climb a vertical tunnel for food, and run in a running wheel. In addition, we trained each functionally active animal for 10 minutes daily to swim, walk on a tight wire, run on several types of running wheels, and climb vertical poles of different diameters. In contrast, physically inactive littermates were raised together in a small cage, where their movements were severely restricted, allowing only enough movement for gaining free access to food and water.

At 35 days of age, each animal was weighed and anesthetized with ether, and their brains were removed and processed according to a modification of the Golgi-Kopsch technique (*12*). After being removed, brains were placed in weighed vials containing a solution of 5 percent glutaraldehyde and 2 percent potassium dichromate, which were weighed again to determine fresh brain weights. Brains remained in that solution for 5 days and were then placed in a 0.75 percent silver nitrate solution for 6 days, after which they were embedded in nitrocellulose. The cerebellar vermis was sectioned in the sagittal plane at 90 μm so that most Purkinje cell dendritic trees lay completely within single sections.

Growth of the body and brain were monitored to assess possible gross effects of physical activity. Body weights of physically inactive mice were 15.5 percent lower, which was consistent with a visually apparent reduction in size, presumably due to a reduction in muscle mass caused by inactivity (Table 1). Whole-brain weights of inactive animals were 3.1 percent lower. This alteration was consistent with reductions in brain weights noted in previous environmental deprivation studies (*6, 13*).

In order to estimate the effects of physical activity on the layers of the cerebellar cortex, we histometrically determined the areas of the molecular and granular layers of three cerebellar lobules in Golgi sections (*14*). All slides were coded so that the experimental conditions were unknown for these and all subsequent measurements. Although no significant difference in the areas of the granular layers was noted between active and inactive animals, the area of the molecular layer was 10.7 percent less in inactive animals (Table 1). This reduction in area was primarily due to the reduced thickness of the layer, which was 9.7 percent thinner than in functionally active mice. Decreased cortical depth has been seen previously in the visual system after environmental deprivation (*15*).

We next examined the dendritic trees of 59 Purkinje cells from active animals and 62 cells from inactive ones. Simple visual examination revealed no qualitative differences in the dendritic trees. Dendritic trees of Purkinje cells of both groups of animals were distinctly planar and oriented in the sagittal plane at right angles to the long axes of the folia of the vermis. No alteration in shape or orientation of the dendritic trees was noted. Measurements of branching density [cumulative length of dendrites per unit area of dendritic field (*16*)] showed no differences between Purkinje cells of active and inactive animals; however, measurements of dendritic field areas (*17*) revealed that Purkinje cells were 9.6 per-

Table 1. Differential effects of activity and inactivity on mice. Mann-Whitney U tests were used to determine the significance of differences; N.S., not significant.

Measure	Active ($N = 6$)	Inactive ($N = 5$)	Difference (%)	$P \leq$
Body weight (g)	15.53	13.12	−15.5	.02
Brain weight (g)	0.415	0.402	− 3.1	.001
Granular layer section area (mm^2)*	0.163	0.168	+ 3.0	N.S.
Molecular layer section area (mm^2)*	0.205	0.183	−10.7	.015
Purkinje cell				
Branching density (mm/mm^2)	448.4	449.1	+ 0.2	N.S.
Dendritic field area (mm^2)	0.0156	0.0141	− 9.6	.001
Total branch length (mm)	6.98	6.33	− 9.3	.009
Spine density (number per row per 10 μm)	9.05	7.65	−15.5	.009
Total spines (number per row per cell)	6348	4906	−22.7	.002

*Means of lobules III, V, and X.

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cent smaller in inactive animals (Table 1). With these direct measurements of dendritic field areas and branching density, we calculated total branch lengths, which were reduced by 9.3 percent in inactive animals (Table 1). In order to determine whether this difference was confined to certain cerebellar lobules, we computed the mean total dendritic branch length per cell for each lobule of the cerebellar vermis and plotted the total branch length of each Purkinje cell in each lobule (Fig. 1). Physical activity had a consistent effect on Purkinje cells throughout the vermis. The difference in total branch length was 11.2 percent if the cerebellar lobules were weighted equally (Fig. 1), which was comparable to the 9.3 percent difference based on animal means (Table 1). This finding also tends to rule out the possibility that the differences observed in total branch length of Purkinje cells between active and inactive animals were due merely to a nonrandom sampling of Purkinje cells of different sizes in different lobules of the cerebellum.

In order to estimate whether physical activity altered the number of potential postsynaptic sites on Purkinje cells for parallel fiber synapses, we counted the number of dendritic spines per unit length of the Purkinje cell dendrite (*18*). Spine density (number per 10 μm of length) was 15.5 percent lower in inactive animals. Similar reductions in spine density in the visual cortex have been reported to follow visual deprivation (*3*), whereas an increase has been noted after sensory stimulation (*4, 19*). From our data on spine density and total branch length, we were able to compute the mean total number of spines on Purkinje cells, which was lower in inactive animals, both as a group and in all littermate comparisons.

From previous research (*7*), we can deduce that the smaller Purkinje cells of inactive animals are not merely cells retarded in an immature form. Immature Purkinje cells have a greater branching density and a greater spine density than those of inactive animals, which seem to be morphologically altered rather than developmentally retarded (*7*). Our previous research (*8*) indicates also that the cerebellar alterations cannot be attributed to nutritional differences. In undernourished mice, both the cerebellar granular and molecular layers are reduced in thickness and Purkinje cell spine density is unchanged; in physically inactive animals, only the molecular layer is thinner and Purkinje spine densities are reduced (*20*). Finally, the differences in body

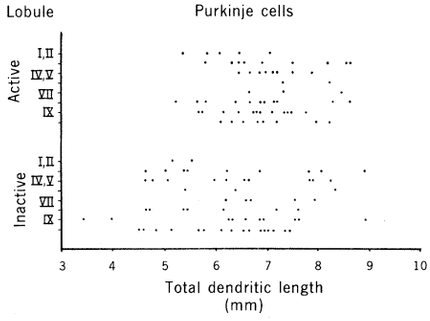

Fig. 1. Purkinje cells of active and inactive animals plotted according to total branch length and location in the cerebellar vermis.

weight between active and inactive animals cannot account for the Purkinje cell alterations since no correlation was found between weights and Purkinje cell total branch length.

Previous observations allow a consideration of the possible cellular mechanisms mediating the altered development of Purkinje cells in inactive animals. The physical presence of afferents plays an important role in the development of the final size and orientation of the Purkinje cell dendritic tree; in the absence of either climbing or parallel fibers, the total dendritic length of the Purkinje cell dendritic tree is reduced (*21, 22*), presumably because of diminished inductive influences for growth. It is only a small extension of these observations to suggest that reduced afferent nervous activity (*23*) can diminish the induction of dendritic growth and spine formation. In addition, rats raised in deprived environments have a smaller amount of neuroglia in the cerebral cortex (*15*) and cerebellum (*6*). A decrease in glial volume could provide for a reduced physical substrate for the growth of Purkinje cell dendrites and contribute to the reduced thickness of the cerebellar molecular layer in inactive animals. It would be of interest to determine whether functional inactivity reduces the number of actual synapses (*24*) and the morphology of spines (*25*) on Purkinje cells.

Two major observations derive from this study. First, even under conditions of severe functional motor deprivation, Purkinje cells are capable of developing most of their normal dendritic tree and most of their normal complement of dendritic spines. Therefore, the development of the Purkinje cell is largely controlled by intrinsic (genetic) and microenvironmental factors such as the physical presence of afferent axons (*22*). This interpretation is in agreement with the observations that synapses are formed in cell cultures kept electrically silent by treatment with local anesthetics (*26*). Second, physical motor activity and its concomitants of enhanced nervous activity (*23*) can produce a small but measurable alteration in the development of the Purkinje cell; total dendritic length and numbers of spines are reduced in inactive animals. These changes in Purkinje cells were accompanied by a selective reduction in the thickness of the cerebellar molecular layer (*27*). The findings are consistent with the hypothesis that the final size and number of spines of the Purkinje cell dendritic tree can be modulated by functional activity.

We have presented evidence that the development of the cerebellar molecular layer and the Purkinje cell are plastic and modifiable by motor activity. It is reasonable to expect that the development of other motor neurons and motor behavior may be alterable also by experience. Specific kinds of exercise have been shown to lead to an earlier onset of walking in human infants (*28*). The effects of functional activity upon the development of dendrites and spines on motor neurons could represent the neuromorphological basis for such behavioral effects of human experience.

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17. Dendritic field outlines were drawn at a magnification of 278 by the camera lucida technique, and dendritic field areas were determined by planimetry.
18. Dendritic spines, in the same focal plane on dendritic shafts, were counted under oil immersion at ×2000 magnification. A minimum of ten spine counts at least 10 μm long, distributed randomly over proximal and distal dendrites, were made for each cell. Ten cells per animal were used.
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23. We would expect that the increased motor activity produced by our treatment would be accompanied by enhanced activity of numerous inputs into the cerebellum including vestibulocerebellar, oblivocerebellar, reticulocerebellar, spinocerebellar, and pontocerebellar systems.
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Holographic Assessment of a Hypothesized Microwave Hearing Mechanism

Abstract. *Exposure of the head to pulse-modulated microwaves induces the perception of a sound. It has been hypothesized that the electromagnetic energy is converted to acoustic energy in the skull and then conducted through the bone. Dynamic time-averaged interferometric holography showed that the predicted motion of head tissue did not occur. An alternative locus for this hearing effect is suggested.*

A person exposed to pulse-modulated microwave energy can perceive the effect as a sound, such as a buzz (*1-3*). The mechanism for the perception is unknown, although it does not appear to be located in the brain itself (*4*).

Foster and Finch (*5*) and Chou *et al.* (*6*) concluded that the electromagnetic energy is transduced into acoustic energy by thermoacoustic expansion in the muscle or bone of the head. In the mechanism they suggested, thermoacoustic expansion would generate acoustic waves that would be conveyed via bone conduction to the tympanic membrane and middle ear. There are many mechanisms of bone conduction (*7*), and the one involved in this process was not specified.

The thermoacoustic expansion-bone conduction hypothesis is based on a study of acoustic transients induced by the impingement of microwave pulses on water in a tank, with the water representing an approximation of the head, and a hydrophone an approximation of the middle ear and cochlea. Support for this hypothesis came primarily from inferences drawn from studies of cochlear microphonics (*6*, *8*).

The thermoacoustic expansion-bone conduction hypothesis is attractive for its apparent simplicity and because it is based on a well-known physical phenomenon (*9*). However, when one considers that ordinary auditory perception is not fully understood and that there are multiple mechanisms for bone conduction (*7*), one finds reason to question this hypothesis. What is needed is a direct physiological test that would show whether or not the skull, or the soft tissue of the head of a mammal, actually shows an acoustic wave when exposed to pulses of electromagnetic energy.

In the experiment described here we attempted, by use of dynamic time-averaged interferometric holography, to find the predicted motion in hair, skin, muscle, bone, and brain in the rat and the guinea pig. This well-established nondestructive testing technique is commonly used in the study of acoustic waves in material. Double-pulse holography was not used since it requires the use of high-power laser pulses that in themselves can induce an acoustic wave in the head and be a confounding variable (*10*).

The time-averaged holography technique consists of making a single holographic recording of an object in which vibratory motion has been induced. It requires that the exposure time in recording the hologram be long compared to one period of the vibration cycle. The hologram effectively stores an array of data representing the time average of all positions of the vibrating object. Where the displacement of the object is zero, the reconstructed image intensity will be brightest. Areas of the object that move will be dim or black in the holographic image. The technique provides information about the amplitude of vibration and the location of vibratory nodes, and is applicable to nonsinusoidal motions. The sensitivity to motion is 0.06 μm. Von Bekesy's data (*11*) concerning vibration of the head in a sound field and its role in hearing by bone conduction, and the analysis by Naftalin (*12*) of hearing thresholds, indicate that the technique would be sensitive to the hypothesized motion if it existed.

The animal was placed on its abdomen on a surface of Eccosorb FR-340 microwave energy absorber placed on the surface of a plywood table that was designed to be isolated from vibration. In some tests, the microwave energy absorber was replaced with a cement block. The microwave energy was generated by a pulsed triode source and directed, by means of a horn antenna mounted over the table, toward the surface of the table. Energy densities were measured before and after each session at the location of the animal's head with a half-wave dipole antenna (*13*).

An etalon-equipped krypton ion laser was used in a standard off-axis holography setup. The exposures of the Kodak S0253 film were controlled with an automatic shutter, and the films from each run were developed together. The coded sets of holograms were assessed blindly by two experimenters using both a Holographics Corporation model 1020 viewer and the diverged beam of a HeNe laser. The holographic setup was tested in several ways to verify that our technique was comparable to that used by others in nondestructive testing of materials (*14*).

Two series of physiological tests were run, one with guinea pigs and the other with rats. A treatment-by-subject design was used in which each animal was its own control. Because of the sensitivity of the holographic technique, all subjects were injected intraperitoneally with an overdose of sodium pentobarbital and the hair over the area of interest was clipped short and a depilatory applied. Experimentation started as soon as there was no detectable heartbeat or respira-

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