of glucose on calcium handling by the islet cells being the fundamental mechanism for stimulation of insulin release (*19*). The present study suggests that the effect of glucose on cyclic AMP synthesis may be secondary to its effect on the intracellular concentration of calcium. Since cyclic AMP itself facilitates insulin release, in part at least by causing an intracellular redistribution of calcium (*2*), the interaction between calcium, calmodulin, adenylate cyclase, and cyclic AMP in islets exposed to glucose would be well suited for amplification of the secretory response.

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12. The specific binding of ^{125}I-labeled calmodulin with 4.0 m*M* $CaCl_2$ (2.76 ± 0.25 pmole/mg) was not significantly different from that found with 1.0 m*M* $CaCl_2$ (2.30 ± 0.06 pmole/mg).
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8 June 1979

Cerebellar Plasticity: Modification of Purkinje Cell Structure by Differential Rearing in Monkeys

Abstract. *Dendritic branching in Purkinje and granule cells and the diameters of Purkinje cell somas were compared in several cerebellar areas of monkeys reared in isolation, with social experience, or in a large colony. In the colony-reared monkeys, spiny branchlets of Purkinje cells were more extensive in the paraflocculus and the nodulus than they were in the other two groups. Granule cell dendritic branching in the paraflocculus and nodulus did not differ across groups. In addition, Purkinje cell somas were larger in the uvula and the nodulus of the colony animals than in the other groups. These data indicate that the social and physical environment during development influences the morphology of cerebellar Purkinje cells.*

The cerebellum, a brain structure involved in coordination of movements, is vulnerable to postnatal trauma such as malnutrition (*1*) and x-irradiation (*2*), yet little, if any, evidence indicates that postnatal behavioral experience can modify cerebellar organization. According to a number of theoretical positions, cerebellar plastic changes should occur. For example, neural models of cerebellar functioning, such as that of Marr (*3*), predict that changes in the strength of cerebellar circuitry underlie the storage of learned motor sequences. Postnatal acquisition of skills requiring motor coordination is a continuing process, as any adult who has learned to drive a car can attest. Since the cerebellum plays a role in the execution of these learned skills, and since changes in cerebellar neuronal function correspond to the acquisition of a motor skill (*4*), the fine structure of the cerebellum may reflect the extent to which the rearing environment allows motor skills to develop.

Behavioral theorists have also argued for the effects of experience on the cerebellum. In particular, Prescott has suggested that abnormal cerebellar development, resulting from restricted social and maternal experience, might underlie aspects of the "primate isolation syndrome" (*5*). The syndrome, which is seen in monkeys after prolonged periods of isolation early in life, consists of a variety of abnormal, autistic behaviors, including social and sexual dysfunctioning as well as movement disorders (*6*). Postulated abnormal cerebellar development associated with this syndrome could possibly reflect the involvement of the cerebellum in social-emotional behavior (*7*).

Numerous studies in rodents, cats, and primates have shown that the fine structure of various brain regions can be altered by experience in development. For example, the extent of neuronal dendrites and the number of spines, the postsynaptic elements of one type of synapse, can be affected by the type and amount of experience during development (*8*). In general, these changes have been demonstrated primarily in forebrain structures such as the hippocampus and neocortex. Such anatomical changes may be involved in or mediate behavioral effects of experience. To date, similar studies have not been performed on hindbrain cortical structures such as the cerebellum. To examine possible anatomical plasticity in the cerebellum, the morphology of two cerebellar cell types was compared in monkeys reared under different conditions. We now report evidence for experience-dependent plasticity in the anatomy of cerebellar neurons (*9*).

Sixteen monkeys, *Macaca fascicularis*, were semirandomly assigned (*10*) to and reared for the first 6 months of life under one of three conditions. Six monkeys, three males and three females, reared in isolation (I) had a very limited sensory and motor environment, having been enclosed in a 1-m^3 Plexiglas cube contained within a sound-attenuating vault (*11*). The I monkeys neither saw nor had physical contact with another monkey during rearing, and there was little in the cage to encourage manipulation or play (*11*). Six monkeys, five males and one female, reared under social conditions (S) were housed in wire cages also about 1 m^3 in size. Cages for pairs of monkeys were adjacent, and 4 hours of play were allowed each day be-

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Table 1. Means and standard errors (S.E.M.) for the various measures in the three rearing groups. Branchlet and cell data are intersections with concentric rings; soma data are cell diameters in micrometers. See text for abbreviations C, S, and I.

Area	C		S		I	
	N	$\bar{X}$ ± S.E.M.	*N*	$\bar{X}$ ± S.E.M.	*N*	$\bar{X}$ ± S.E.M.
Spiny branchlets						
Ventral paraflocculus	2	40.0 ± 1.9*	4	32.9 ± 1.1	2	36.1 ± 1.7
Nodulus	2	26.5 ± 1.3†	4	25.4 ± 0.8	2	24.6 ± 1.3
Flocculus	4	31.6 ± 1.1	6	30.2 ± 1.0	6	32.8 ± 1.1
Granule cells						
Ventral paraflocculus	2	33.9 ± 1.4	4	35.5 ± 1.3	2	34.3 ± 1.3
Nodulus	2	45.4 ± 1.9	4	45.7 ± 1.3	2	44.1 ± 1.5
Purkinje somas						
Nodulus	2	33.2 ± 0.2*	3	29.8 ± 0.2	2	30.8 ± 0.2
Uvula	2	30.8 ± 0.3*	4	28.8 ± 0.5	2	28.6 ± 0.3

*$P < .05$. †$.05 < P < .10$.

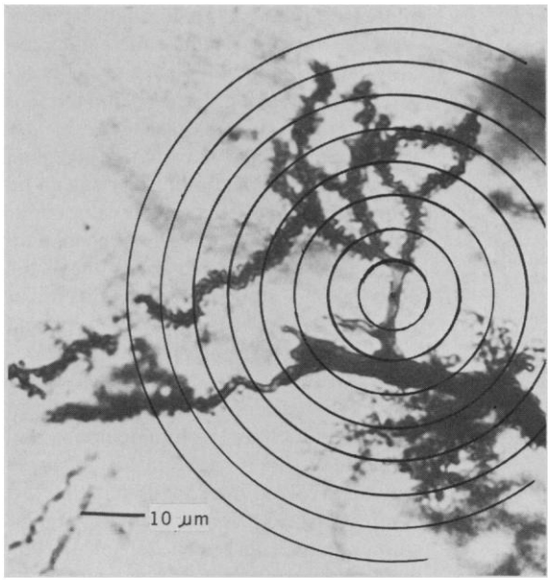

Fig. 1. Golgi-Cox-stained spiny branchlet units were quantified by counting the number of dendritic intersections with concentric spheres 5 μm apart.

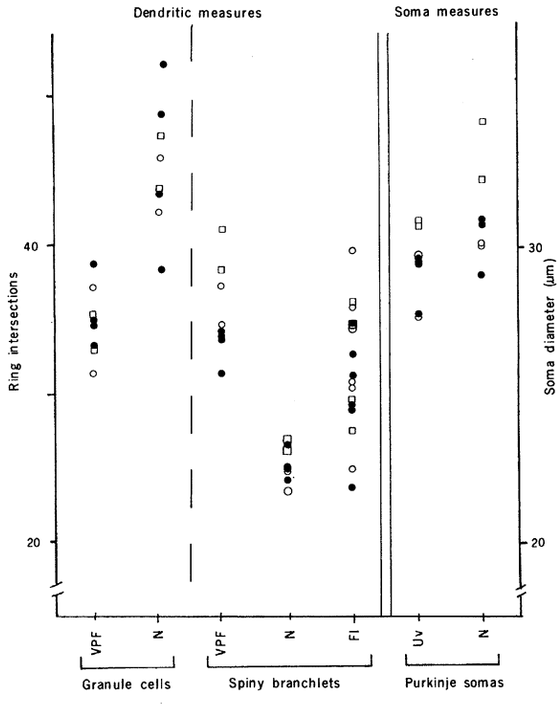

Fig. 2. Means for individual animals. Symbols: □, colony-reared monkeys; ●, socially reared; and ○, isolation-reared. Abbreviations: *VPF*, ventral paraflocculus; *N*, nodulus; *Fl*, flocculus; and *Uv*, uvula.

tween pairs of monkeys. Social monkeys also had the opportunity to see and hear other monkeys as well as people, as there were several monkeys housed in this manner in the same room. Four colony-reared monkeys (C), two males and two females, were housed in a semi-naturalistic setting—two interconnected large rooms with monkeys of all ages and both sexes. Several large fixed structures and smaller manipulable toys were present for play. Thus the three rearing conditions varied both the social and the physical complexity of the environment.

At 8 months of age, after behavioral testing (*12*), all animals were killed and the cerebella removed and stained by the Golgi-Cox method (*13*). At this point, all tissue was coded so the person collecting the data was unaware of the treatment condition of individual animals or slides. Portions of the dendritic tree of the Purkinje cell neuron of the cerebellum, termed spiny branchlet units (*14*) (Fig. 1), were traced and analyzed with a computer-assisted microscope system (*15*), which allowed dendritic fields to be analyzed in three dimensions. Entire granule cell dendritic fields were also analyzed with this system. Variability in staining allowed accurate analysis of various cerebellar areas of subsets of the 16 animals. Three cerebellar areas were chosen for dendritic quantification: ventral paraflocculus, nodulus, and flocculus. Twenty spiny branchlet units were analyzed from each area of each animal, and 20 to 30 granule cells were analyzed for each animal from the paraflocculus and the nodulus. A three-dimensional concentric-ring analysis (*16*) was applied to each cell and unit. In this analysis, intersections between concentric spheres surrounding the spiny branchlet origin or granule cell body and dendritic branches were counted (Fig. 1). This technique provided a quantitative estimate of the linear amount of dendrite. The distance between adjacent spheres was 5 μm for Purkinje branchlets and 2 μm for granule cells.

Cresyl violet staining of aldehyde-fixed material (*17*) was used in two cerebellar areas, the uvula and the nodulus, in order to measure the size of Purkinje cell somas. Between 100 and 200 somas were drawn at a magnification of 500 from each of these two areas for each animal with the aid of a camera lucida. These drawings were then used for hand measurement of the soma "diameter," the widest portion of the cell body in the plane parallel to the pia mater (*18*).

These three measures were analyzed with the exact randomization test (*19*),

which was applied to the means for individual animals, a more conservative procedure than treating each neuron and spiny branchlet as an independent measure. Both males and females are represented in each group for all comparisons. Rearing environment had a statistically significant effect in the paraflocculus, a late-developing cerebellar area (*20*); the nodulus, an earlier-developing area (*20*); and the uvula (Table 1). There was no significant sex difference in any area (Mann-Whitney U test), and no significant differences were detected in dendritic branching of granule cells (exact randomization test).

Thus parafloccular, nodular, and uvular areas are consistent in showing larger, more complex Purkinje cells in those monkeys reared in seminaturalistic conditions. The absence of positive correlations with body weight (*12*), as well as the appearance of the effects in Purkinje but not granule cells, suggests the effects are not due to overall growth differences among individuals. However, floccular spiny branchlets do not show this general pattern. Although the mean for I monkeys is highest as a group, individual animal means are scattered (Fig. 2). Except for the flocculus, these data indicate that the anatomy of the developing cerebellum is plastic in the sense that the soma size and dendritic morphology of cerebellar cells is affected by characteristics of the environment in which the monkey is reared. Moreover, this difference appears to occur in a particular cell population, the Purkinje cells, and not in granule cell dendrites. Presumably a net increase in granule cell axon extent or in the frequency of contacts with Purkinje spiny branchlets does occur with increased extent of spiny branchlet material. Cerebellar areas seem to differ systematically within animals. Spiny branchlets in the paraflocculus were larger than those in the nodulus, although Purkinje somas in the nodulus tended to be larger than those in the uvula. Dendritic branching of nodular granule cells appeared to be greater than in those of the paraflocculus. Floccular measures of spiny branchlets were highly variable. This effect seems to hold across all rearing conditions; within individual areas, C monkeys tended to have larger Purkinje somas.

The neuronal organization of the cerebellum is similar from fish to humans, a consistency through vertebrate phylogeny that has contributed to doubt that the cerebellum might be modifiable by experience. This evidence for plasticity in cerebellar circuitry indicates that phylogenetically stable structures, as well as phylogenetically newer ones, are not "hardwired" and can be modified by environmental experience. It is of interest that the cerebellum, like the hippocampus, matures late. The paraflocculus is among the latest developing of cerebellar structures, and the nodulus is among the earliest (*20*). Sensitivity to experience may be a function of the maturity of the structure at the time of the experience. It is possible that both the within-animal differences and the experiential effects reflect different times of maturation of the areas studied. Additionally, the cerebellum, like the neocortex, is a structure that integrates a variety of incoming information into an appropriate output. In terms of both maturation and function, plasticity of the cerebellum is perhaps no more surprising than the earlier demonstrations of plasticity in the hippocampus or neocortex.

The locus of plasticity in Purkinje cells, but not in granule cell dendritic branching, is compatible with Marr's (*3*) model of cerebellar function in which he proposed changes in the spine synapses of the Purkinje cell to store learned motor sequences. Granule cells, in his parsimonious scheme, need not undergo such changes in order to account for cerebellar learning.

The data from these cerebellar structures do not support Prescott's hypothesis (*5*) that cerebellar changes might underlie primate isolation syndrome behaviors, since differences were not found between monkeys reared in isolation and monkeys reared with social contact. Although monkeys reared in these two conditions behaved differently (*6*, *12*), these differences were not reflected in any anatomical measures. Though I-group means sometimes exceeded S-group means, this pattern was not consistent; and individual I and S monkeys varied and overlapped (Fig. 2). Instead, the monkeys reared in an environment that provided more opportunity for physical activity differed from these two groups. Of course, isolation-induced functional changes may not be reflected in anatomical measures or may be mediated by unstudied cerebellar areas or other brain regions. Cerebellar plasticity as assessed by these measures, however, reflects the complexity of the physical environment rather than the mere presence of social contact.

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9. Ours is one of two studies that simultaneously reported this phenomenon [M. K. Floeter, W. T. Greenough, G. P. Sackett, *Soc. Neurosci. Abstr.* **4**, 471 (1978); G. M. Weiss and J. J. Pysh, *ibid.*, p. 481]. See also J. J. Pysh and G. M. Weiss, *Science* **206**, 230 (1979).
10. Constraints were imposed by balancing groups by sex, dates of birth, and so forth.
11. Animals were placed in a translucent bag when being removed for weighing and routine maintenance. Both I and S cages contained a heating pad for the first 3 months, a diaper for the first 4 months, and a built-in section of 10-cm plastic pipe, from which the drinking tube protruded. The sound level in the colony room was 71 dB measured on the A scale), and in the chamber, 40.5 dB.
12. Behavioral tests indicated much higher frequencies of abnormality (for example, rocking, stereotypy, aberrant social interactions) in I monkeys than in the other two groups (W. T. Greenough, G. Sackett, K. Wrege, in preparation). Terminal body weights were not taken for all animals. Mean body weights at 6 months of age were C, 0.973 kg; S, 1.119 kg; and I, 1.064 kg.
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14. A spiny branchlet "unit" was defined as a branched portion of the Purkinje cell dendritic tree which emerged at a single point from a smooth branch. No correction was made for occasional branches that traveled off the section.
15. The microscope system allowed the operator to move the stage in 0.5-μm steps in all three dimensions with the aid of a computer (Nova 3). The computer stored, at operator command, three-dimensional coordinates of a point along a dendrite and an identifying label (such as "point along branch" and "bifurcation"). The three-dimensional structure of the neuron could then be analyzed by a program that calculated the number of dendritic-ring intersections (*16*).
16. D. A. Sholl [*The Organization of the Cerebral Cortex* (Methuen, London, 1956)] proposed the use of concentric rings for two-dimensional analysis of drawings. This was extended to three-dimensional concentric spheres.
17. Frozen sections were cut at 30 μm in the plane transverse to the longitudinal axis of the folium, mounted on gelatinized slides, and stained with cresyl violet.
18. For the uvula and nodulus, equal numbers of cell somas were collected from the convexities, concavities, and sides of folia.
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26 December 1978; revised 29 May 1979