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Early Experience Effects upon Cortical Dendrites: A Proposed Model for Development

Abstract. *We studied the effects of environmental stimulation on the development of rat cortical pyramidal cell synaptic loci (dendritic spines) and the number of such cells staining by the rapid Golgi technique. Stimulation three to five times a day from the day of birth increased the number of spines per micrometer in 8-day-old animals and increased the number of neurons staining at 8 to 16 days of age. This effect of afferent input upon development of the dendritic spine may represent the neuroanatomical basis for the influence of early experience on subsequent behavior. The number of neurons staining by the rapid Golgi technique appears to be related to those that are functionally involved at the time of tissue preparation.*

It is generally accepted that environmental influences during certain periods of early postnatal life have an effect upon later development and behavior (*1*). The mechanisms whereby early experience induces such long-lasting, and perhaps permanent, effects upon the behavior of the organism are unclear. Hebb has suggested (*2*) that the richness of neuronal interconnections represents the neuroanatomical substrate of behavior. As functional contact between two neurons is established when the presynaptic elements of one axon make contiguous contact with the postsynaptic elements of an adjacent dendrite, it seemed possible that the developmental patterning of such synaptic connections may underlie, or be related to, later behavioral flexibility.

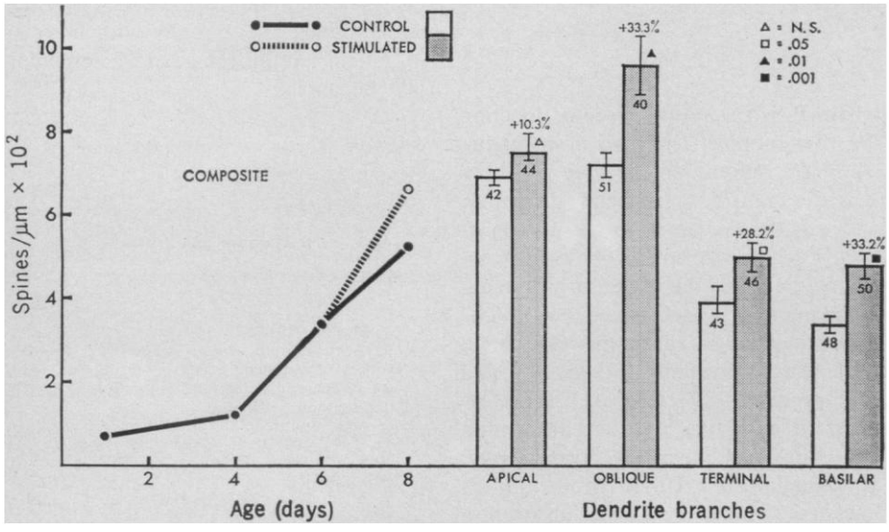

Fig. 1. Effects of environmental stimulation upon spine development on four different dendritic branches of the cortical pyramidal cells. Animals were treated as described. The numbers in the bar graph are the numbers of neurons from which the indicated values for dendritic spines on the various branches were determined. Animals are 8 days old. The composite graph illustrates the average number of spines per micrometer of all dendrite branches covered in the four dendritic locations specified. All values shown for dendrite branches include S.E.'s and are derived from five control and five stimulated animals.

Electron microscope studies have demonstrated that the dendrite spine is a distinctive feature of the postsynaptic apparatus in cerebral cortex, cerebellum, brain stem, and spinal cord (*3*). These lateral extensions of the dendrites were first described by Cajal (*4*) and, although initially considered an artifact, are now recognized as representing "specific postsynaptic receptive structures on the dendrites" (*5*). The cortical dendrites of the neonatal kitten do not have spines, and the few qualitative studies performed (*5*) indicate that they appear slowly during development in association with increasingly complex behavioral and neurophysiological changes. In the rat, spines are also absent at birth and only obtain adult values shortly after weaning (*6*). The identity of these structures with functional units of the central nervous system (the synapse) and their gradual appearance during development suggest that their ontogeny may in some manner be related to later behavior.

Decreased visual stimulation (*7*) or interruption of the visual pathway (*8*) during periods of spine development results in a decreased number of spines in the adult animal, and handling during the early postnatal period increases the rate of formation of new cells in the cerebellum (*9*). Other investigators (*10*) experimenting with sensory-enriched or sensory-deprived environments have concentrated upon manipulating the environment after weaning. The rat brain at this time is essentially mature as judged by biochemical, neurophysiological, and morphological criteria (*11*), and its period of plasticity is largely over. It was suggested earlier (*12*) that sensory input during maturation of the dendrite spines may accelerate their development and that this differential acceleration of specific neuron processes with their interconnections may represent the neuroanatomical basis for the effects of early experience on subsequent behavior. We designed an experiment to test this hypothesis by initiating diverse sensory stimulation prior to weaning, during the period of central nervous system (CNS) maturation.

Separate litters of Sprague-Dawley rats bred in this laboratory were used. From the day of birth until they were killed at 8 days of age, random litters were either left untouched or were re-

moved from the nest gently with forceps three to five times a day and subjected to a wide range of sensory stimuli for 20 to 30 minutes: they were handled and stroked, shaken on a mechanical shaker; placed in warm and cold water and on cold and then warm metal; and subjected to noise and flashing lights and to short periods of electric shock. At 8 days of age entire litters were killed, and a 3-mm wedge of brain, through the visual and auditory cortex, was removed and placed in a solution of osmic acid and potassium dichromate. Each litter was either handled or left undisturbed. Two or three brains from each handled group and from each undisturbed group were chosen at random for histological studies. Additional normal infant rats, raised with no attempt to limit sensory experience, were killed on days 1, 4, and 6 after birth, and brains were fixed as described and sectioned. The sections were stained by a variant of the rapid Golgi technique used for quantitative determination of the number of spines on cortical pyramidal cells (*8*). After a 3-mm cortical wedge was fixed and then stained with silver nitrate, 100-μm sections were cut parallel to the surface and were washed in absolute alcohol, methyl salicylate, and xylene. They were then mounted under neutral synthetic resin. Slides were coded, and all counts were tabulated as unknowns at $\times$ 450 magnification. Five control rats and five experimental (stimulated) rats were used; a minimum of eight neurons were studied on each animal. Spines were counted at four different sites on each neuron, as described by Globus and Scheibel (*8*). Lateral extent (in micrometers) of dendrite growth, apical dendrite length from the cell body, and widest and longest cell body dimensions were also measured. A further experiment was designed to determine whether the small percentage of neurons visualized by the rapid Golgi staining technique might in some manner be related to the neurons that were functionally unique when, or just before, the animals were killed and the tissue was prepared. Two separate series of animals were stimulated several times a day or left undisturbed from the day of birth until they were killed. The first series was killed on days 8, 11, 12, and 16, and the second series on days 9, 11, and 13. Stimulated animals were killed immediately after the extra handling routine; brains were stained and sectioned as described. The slides were coded, and the stained pyramidal neurons in all sections derived from each cortical wedge were counted and divided by the number of sections; the average number of stained neurons per section was thus determined.

Table 1. Effect of increased stimulation upon the number of cortical pyramidal cells visualized by the rapid Golgi technique. In column 2, C represents control groups and S represents stimulated groups; three animals were treated in each case except where otherwise indicated. In the first experiment (series A), statistical analysis was not possible because individual slides were not tallied separately within each treatment group. In the second experiment (series B), done several months later, the stained cells in each cortical section were tabulated individually. Data shown are with S.E.

Age (days)	Treatment	Sections (No.)	Cells (No.)	Cells per section
		Series A		
8	C	44	754	16.94
8	S	40	985	24.62
11	C	59	1479	24.86
11	S	62	1996	31.94
12	C	35	983	26.66
12	S*	38	1199	31.56
16	C	73	942	12.82
16	S	71	1055	15.10
		Series B		
9	C	72	1453	20.18 $\pm$ 2.92
9	S	67	2525	37.69 $\pm$ 4.82‡
11	C	74	1850	25.00 $\pm$ 2.19
11	S	75	2688	35.84 $\pm$ 3.92§
13	C	55	706	12.84 $\pm$ 1.08
13	S†	74	1439	19.45 $\pm$ 1.44‡

* Two animals. † Four animals. ‡ Difference between stimulated and control significant at 0.001 level. § Difference between stimulated and control significant at 0.01 level.

Figure 1 indicates that, at each dendrite location studied, the stimulated animals had more spines per micrometer than did the nonstimulated control animals. It is also of interest that this increase in spine density took place without any change in cell body dimensions or in apical or lateral dendrite length (control versus handled animals: width, 16.0 $\pm$ 0.03 versus 16.4 $\pm$ 0.2 μm; length, 23.3 $\pm$ 0.6 versus 24.2 $\pm$ 0.4 μm; apical length, 351 $\pm$ 7 versus 360 $\pm$ 13 μm; lateral extent, 83 $\pm$ 2 versus 85 $\pm$ 3 μm).

According to Globus and Scheibel (*8*), the "specific (visual) afferent radiation, in rabbit at least, is applied directly to the apical dendrite of pyramids." They suggest that the basilar and oblique dendrite branches may subserve collaterals arriving from "another powerful afferent system." However, Anokhin (*13*) and Purpura (*14*), after studying the ontogeny of the evoked potential, conclude that during early development specific radiations synapse on the dendrite branch in layer IV, whereas the "synaptic pathways related to apical dendrites of cortical pyramidal neurons are primarily involved in the production of a major portion of the . . . nonspecific evoked activities" (*13*). Our results indicate that the predominant effect upon the neonatal rats of increased environmental stimulation was to increase the number of countable spines on the basilar and oblique dendrite branches; terminal branch spines were less affected, and apical spines were increased slightly (*15*). These results suggest that sensory stimulation during the period of spine growth may differentially accelerate the maturation of synaptic loci on the pyramidal cell dendrite tree (*16*). The stimulated animals had more neurons staining at all ages examined (Table 1). This effect may be related to the increased number of neurons functionally and, therefore, metabolically involved. Perhaps the small percentage of neurons generally staining with the rapid Golgi technique is related to those neurons that are unique functionally, and therefore metabolically, before the animal is killed.

As the sensory stimuli we used were deliberately multidimensional, we cannot at this time determine whether the differential effects on spine development and neuron visualization represent specific or nonspecific afferent influences upon the developing neuron. At 8 days of age spine density is changing rapidly and is 25 to 40 percent of the adult values achieved at approximately 21 days of age (*6*). This period in the development of the CNS is particularly dynamic (*11*). We cannot conclude that the total numbers of spines in adults will be different in the stimulated animals; we are concerned here only with rates of development. Basic CNS patterns and interconnections established during periods of rapid development and differentiation may provide the neuroanatomical foundation upon which more temporary connections are later applied.

The mechanism whereby sensory stimulation accelerates neuronal development is unclear. As a working hypothesis we propose (i) that afferent input during the period of spine development ("critical period"?) increases regional blood flow to the maturing neuron field (*17*); (ii) that this increased blood flow results in an enriched nutritional environment, which provides a competitive growth advantage to the relaying and receiving neurons and their spine processes; (iii) that a differ-

ential development of dendritic spines and their interconnections occurs in response to the localized blood flow and biochemical changes associated with the afferent input; (iv) that the patterning of these interconnections lays the foundation for later behavioral performance. This mechanism may thus provide the neuroanatomical framework underlying the complex of phenomena generally referred to as the influence of early experience on development and behavior.

SHAWN SCHAPIRO
KATHERINE R. VUKOVICH
Developmental Neuroendocrinology Laboratory, Veterans Administration Hospital, San Fernando, California and *Department of Psychiatry, University of California, Los Angeles 90024*

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15. We have since completed another series of experiments in which the spines on the apical as well as basilar, oblique, and terminal dendrite branches were increased by extra stimulation in 9- and 13-day-old animals.
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Rhodanine: A Selective Inhibitor of the Multiplication of Echovirus 12

Abstract. *A search for compounds which have previously unrecognized antiviral activity led to the discovery that rhodanine inhibits the multiplication of echovirus 12 and also the development of virus-induced morphologic changes. Eighteen derivatives and analogs of rhodanine were synthesized and tested against echovirus 12. These compounds were considerably less active than rhodanine or were inactive, and some of them were more toxic to the host cells than rhodanine.*

It is timely to find previously unknown antiviral activities of organic compounds so that new effective inhibitors can be synthesized through guidance of inhibition-structure relationships, as exemplified by the knowledge on the structural requirements for selective inhibitors of enteroviruses by 2-(α-hydroxybenzyl)-benzimidazole and related compounds (*1*).

We have found that rhodanine (2-thio-4-oxothiazolidine) is a selective inhibitor of the multiplication of echovirus type 12. Rhodanine and its derivatives have been tested in diverse biological systems (*2*). 5-(β-Carboxyethyl)-rhodanine has been used as an insecticide for plants (*3*). 3-Methyl-5-(4′-nitrophenylazo)-rhodanine cured dogs which were parasitized by *Ancylostoma caninum* and *Uncinaria stenocephala* (*4*). Tuberculostatic and other bacteriostatic activities in vitro were reported for 3-amino-5-(4′-hydroxy-3′-methoxybenzylidene)-rhodanine (*5*) and 3-arylrhodanines (*6*) and 3,5-dialkylrhodanine (*7*). Soil fungi have been controlled by 3-(2′-cyanoallyl)-rhodanine (*8*). *Aspergillus niger, Botrytis cinerea, Penicillum italium*, and *Rhizopus nigricans* have been controlled by 3-aryl- (*9*) and 5-propylidenerhodanines (*10*). Rhodanine and 5-methylrhodanine have been reported to show antithyroid activity (*11*). Electrical and drug-induced spasms have been responsive to 3-ethyl- and 3-phenylrhodanine (*12*). 5-(α-Furanyl)-rhodanine has been studied for activity against Ehrlich, Yoshida, and sarcoma 180 ascites tumor cells (*13*).

In addition to these diverse biological activities, minimum antiviral activity against Columbia SK virus in mice for piperonylidenerhodanine has been reported by Cutting and Furst (*14*). Consequently, we decided to include rhodanine and its derivatives in our studies on viruses.

The antiviral spectrum of rhodanine was determined in tube cultures of primary kidney cells from the rhesus monkey. The cultures were maintained in 2 ml of Eagle's minimum essential medium which contained different concentrations of rhodanine. About 100 tissue culture infective doses ($TCID_{50}$) of virus were inoculated in 0.1 ml amounts into groups of tubes with and without rhodanine. Virus multiplication was determined by recording the development of virus-induced cytopathic effects (CPE). Inoculated cultures were maintained at maximum for 9 days, depending on the virus used. Details of experimental procedure have been described (*15*).

A virus was considered susceptible to rhodanine if, on any day of the observation period, there was more than 90 percent reduction of virus-induced cell damage in the presence of 150 μg of rhodanine per milliliter as compared to the control cultures. If there was less than 50 percent reduction of CPE at this concentration of rhodanine, a virus was classified rhodanine-insusceptible. Between these extremes, a group of slightly susceptible viruses was established.

Rhodanine at a concentration of 150 μg/ml is nontoxic to monkey kidney cells as judged by microscopic examination; a concentration of 200 μg/ml sometimes causes granulation of cells, but only after exposure for 4 days or more.

The spectrum of the virus inhibitory activity of rhodanine was determined. Only the echovirus 12 was particularly susceptible. Many other picornaviruses, reovirus 3, vesicular stomatitis virus, and three representatives of DNA-containing viruses—adenovirus 7, herpes simplex, and vaccinia virus—were insusceptible. Since herpes simplex virus