Changes in Cortical Dendritic Branching Subsequent to Partial Social Isolation in Stumptailed Monkeys

ROBERT G. STRUBLE
AUSTIN H. RIESEN
Department of Psychology
University of California, Riverside
Riverside, California

Stumptailed monkeys were reared from 1 week after birth to 6 months of age in either a colony condition with the mother or in partial social isolation that allowed visual contact with the colony animals, but not physical contact. At 6 months of age the animals were killed and selected areas of the neocortex stained by the Golgi-Cox method. Relatively nonspiney cells of Layer IV were drawn and analyzed for complexity of dendritic branching. Isolation-reared animals had significantly decreased branching complexity in Motor I cortex when compared to the control animals. A transform of the data that related the number of branches to the number of previous branches showed a slight rearing effect in Somatosensory I cortex with the deprived animals having a lower rate of branching than the controls. We conclude that social isolation also includes a motoric deprivation that could account for these data.

Rearing monkeys from an early age in social isolation has been shown to result in numerous bizarre behaviors (e.g., self-clutching, rocking) and, when the animal reaches adulthood, to interfere with normal behaviors (e.g., sexual and maternal; see Suomi, Harlow, & Kimball, 1971). A tacit assumption made is that these abnormalities are consequent to limitations of the social environment. In oblique opposition to this interpretation is the suggestion of Prescott (1970) that some of the behavioral abnormalities of socially restricted primates are the result of somatosensory restriction. Prescott has emphasized particularly the likelihood of vestibulo-cerebellar dysfunction in the social isolation paradigm. Specifically, he has suggested that some emotional-behavioral disorders are "...directly attributable to deprivation of the somatosensory system" [Prescott, 1970, p. 357]. Support for this position is found in the work of Mason and Berkson (1975) and Eastman and Mason (1975) who have reported that raising a social isolate monkey on a moving surrogate (a cloth-covered cylinder), as opposed to a stationary surrogate, can attenuate some emotional-behavioral disorders.

The present study was performed in an attempt to find changes in neocortical dendritic branching as a consequence of social isolation.

Reprint requests should be sent to Austin H. Riesen, Department of Psychology, University of California, Riverside, Riverside, California, 92521, U.S.A.

Received for publication 6 April 1977
Revised for publication 1 July 1977
Developmental Psychobiology, 11(5):479-486 (1978)

0012-1630/78/0011-0479$01.00

Method

Subjects

The subjects of this study were stumptailed macaques (*Macaca arctoides*) born at the University of California, Riverside primate colonies. Control animals were born in the indoor colony and not disturbed until termination of study except for occasional brief (< 1/2 hr) use in behavioral tests in an open field. Experimental animals were born to mothers of the outdoor colony and placed in the experimental condition on a terry-cloth-covered heating pad after separation from their mother at 1 week of age. The experimental animals were hand-fed until self-feeding occurred, usually at 9-11 days of age. The heating pad was removed after adequate self-feeding was observed for at least a week. These animals were not disturbed except for weekly transfer to a holding cage for home cage cleaning.

Five of the animals were controls (3 male, 2 female) and 4 were experimentals (2 male, 2 female). Preliminary analysis of the data showed no gender differences so that gender was ignored as a factor thereafter.

Procedure

Housing. The experimental condition was a clear Plexiglas cubical cage, 30 cm per side with a steel grid floor. The cage was mounted in the front wall of the indoor colony cage so that the experimental animals could see the whole colony cage and had relatively normal auditory and olfactory experiences. They could not at any time touch or be touched by the colony animals. Experimental animals were fed up to 500 ml of Similac (Ross Laboratories) daily, fresh fruit and vegetables when available, and, beginning at 1 month, Purina Monkey Chow soaked in Similac. The control animals were raised with their mothers in the inside colony cage measuring 3.3 x 2.9 x 4.3 m. The colony consisted of 1 adult male, 4 adult females, 2 adolescents, and 1-3 infants (the control animals). The colony was fed twice daily with Purina Monkey Chow supplemented with fresh fruit and vegetables when available. The daily light cycle was 14 hr lights on/10 hr lights off from May to October, and 12 hr lights on/12 hr lights off the remaining months.

Tissue Preparation. At 6 months of age the monkeys were tested briefly (20 min in an open field; results to be reported in a subsequent paper), weighed, and given 50 mg/kg sodium pentobarbital, intraperitoneally. When deep anaesthesia was reached, the animal was mounted in a stereotaxic instrument and a craniotomy performed. The brain was cut at the level of the posterior pons leaving the cerebellum intact, and lifted from the brain case, weighed, and the relevant brain areas dissected out with a scalpel.

Areas taken for staining included Motor I and II (M-I, M-II) and Somatosensory I and II (S-I, S-II) as defined by Woolsey (1958; p. 68). The visual cortex (V-I) was cut from an area just posterior to the lunate sulcus to within a centimeter of the posterior pole and superior (about 2 mm) from the occipital sulcus for 1.5 cm dorsad. The prefrontal cortex (PF) was defined as the lateral aspect of the frontal lobe 5 mm anterior to the arcuate sulcus and extending rostral to the frontal pole. All pieces were 5-7 mm thick.

Staining (4 weeks in solution), embedding in celloidin, and mounting were done according to the tungstate modification of the Golgi-Cox method using bulk

alkylinization as presented by Ramon-Moliner (1970). The embedded tissue was affixed to a block and cut on a sliding microtome at 120 μm, such that the sections were normal to the pia as evidenced by the apical dendrites. Representative sections from the whole extent of the tissue were kept for analysis. All slides were coded and neither the animal number nor the condition was known by the experimenters. All data derived from these slides were kept in this coded form until final tabulation.

Cells were required to have their cell bodies in the middle of the tissue section and not be obscured by other stained objects (see Fig. 1). Relatively nonspiney stellate cells close to or in Layer IV were selected for drawing to avoid misoriented pyramidal cells and Jones (1975) Type-7 cells. (Layer IV was generally recognized by a paucity of cell bodies.) Second, the distance from pia to white matter was bisected. These 2 methods agreed closely. In M-I, the 2nd method was used exclusively. The selected cells were drawn on paper with the aid of a grid in the microscope occular. All cells were drawn at 500 x. All dendrites within the tissue section were drawn. No discrimination was made between dendrites and axons. The branch scoring method was identical to one used by Coleman and Riesen (1968). First-order dendrites were defined as arising from the cell body, 2nd-order from the branching of a 1st order branch, and so on. Any branches beyond 5th-order were not included in the data analysis. Each cell was checked for accuracy by another observer. From each cortical area 10 cells were drawn and scored. They were then averaged to give a mean score for each branching order for each cortical area for each animal. This mean score was used to compute the branching ratio (see below).

An unequal n, least squares analysis of variance for repeated measures (Winer, 1962) was performed independently for each cortical area. Tukey's HSD test of simple main effects (Kirk, 1968) was performed to delimit significant differences.

Results

Whole brain weights were not significantly different between the 2 groups, weights ranging between 85 and 106 g. Most cortical areas showed only nonsignificant differences (Table 1 and Fig. 2).

The interaction between Rearing Condition and Dendritic Order in M-I was significant ($F = 8.88$, $df = 4/28$, $p < .001$; see Fig. 2). Use of the Tukey HSD test showed that the control animals had a greater mean number of 3rd-order dendritic branches than did the deprived animals.

A transformed variable of the branching scores was made, subsequently referred to as the branching ratio (Br). The Br was formed by dividing the number of branches of the nth-order dendrites by the number of $(n - 1)$th-order dendrites, giving a ratio of branching at each Order-interface. This transform of the data was justified by the following argument: With the obvious exception of the 1st-order dendrites, the number of branches of any order will be related to the previous order's number of branches. The Br would then represent the rate of branching of an order independently from the numbers of branches of previous orders.

In M-I cortex, control animals had a higher overall mean Br than did deprived animals as shown by a significant main effect of Rearing Condition ($F = 7.11$, $df = 1/7$, $p < .05$; see Fig. 3). Rearing also affected some branching ratios at particular

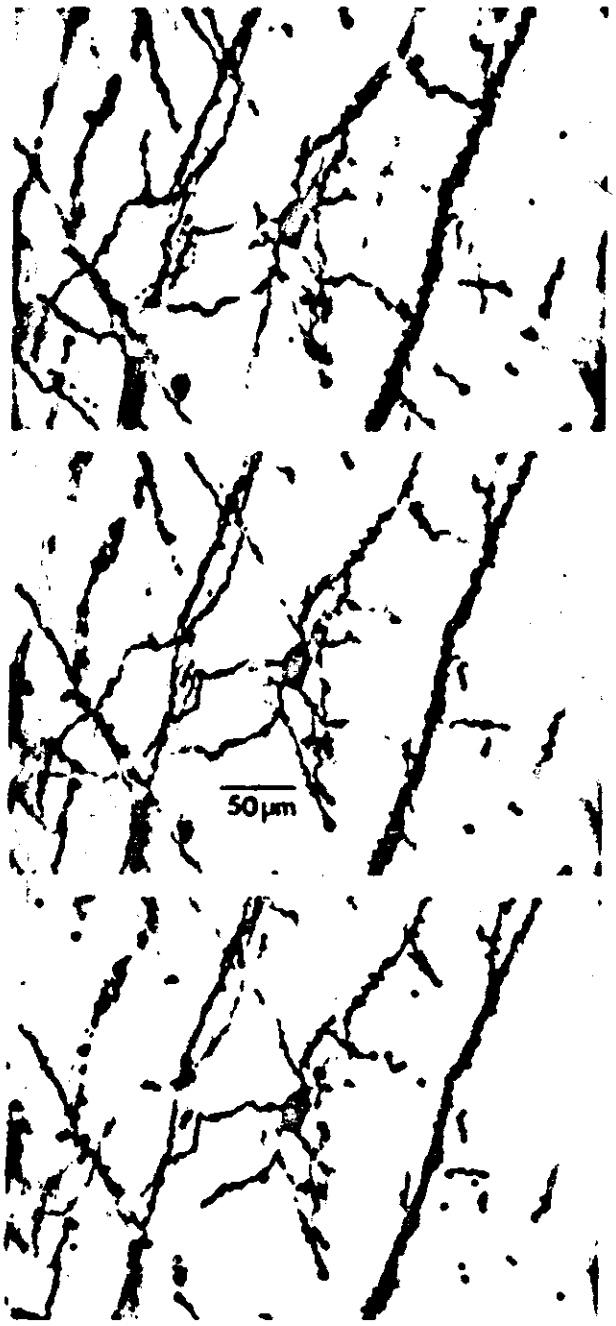

Fig. 1. A stellate cell typical of those selected for dendritic tree analysis is shown centered in photomicrographs differing in plane of focus. The thick process to the right of the stellate cell and extending from top to bottom of each plate is an apical dendrite of a Layer V pyramidal cell. In the top photo the spines of the apical dendrite can be seen most clearly. The large number of spines on the apical dendrite contrasts with the relatively spine-free nature of the stellate cell. Pial direction is to the top.

TABLE 1. Mean (SEM) Branching Scores and Branching Ratios by Cortical Area for Isolated (I) and Colony-Reared (C) Animals.[a]

Area		Mean (SEM) Branches (branch order)					N^b
		1	2	3	4	5	
V-I	I	6.62 (.19)	9.30 (.39)	7.08 (.91)	2.82 (.52)	.75 (.13)	4
	C	6.36 (.27)	9.00 (.66)	7.22 (.46)	2.92 (.48)	.92 (.12)	5
M-I	I	7.32 (.19)	10.60 (.21)	7.22 (.42)	4.20 (.43)	1.80 (.29)	4
	C	6.76 (.21)	10.60 (.38)	9.72 (.61)	5.24 (.47)	2.36 (.46)	5
M-II	I	5.50 (.55)	7.60 (.57)	6.07 (.29)	2.63 (.60)	.53 (.35)	3
	C	5.96 (.50)	8.36 (.42)	7.12 (.51)	3.60 (.47)	1.18 (.19)	5
S-I	I	6.65 (.53)	8.55 (.48)	6.10 (.98)	2.42 (.53)	.90 (.33)	4
	C	6.56 (.15)	9.86 (.15)	7.62 (.44)	2.82 (.25)	.56 (.10)	5
S-II	I	6.78 (.40)	8.98 (.42)	7.55 (.65)	3.32 (.52)	1.25 (.44)	4
	C	6.92 (.24)	9.46 (.26)	8.14 (.58)	3.74 (.17)	1.16 (.15)	5
PF	I	6.77 (.55)	9.40 (1.21)	7.77 (1.27)	4.00 (.12)	1.20 (.23)	3
	C	7.40 (.65)	9.57 (1.52)	8.40 (1.31)	4.17 (.39)	1.33 (.35)	3

Area		Mean (SEM) Branching Ratio (Order-interface)				N^b
		1-2	2-3	3-4	4-5	
V-I	I	1.41 (.09)	.76 (.07)	.40 (.04)	.28 (.07)	4
	C	1.41 (.05)	.81 (.04)	.42 (.08)	.32 (.02)	5
M-I	I	1.44 (.02)	.68 (.03)	.57 (.04)	.42 (.06)	4
	C	1.57 (.02)	.92 (.06)	.54 (.03)	.44 (.07)	5
M-II	I	1.39 (.05)	.81 (.04)	.43 (.10)	.18 (.09)	3
	C	1.42 (.07)	.87 (.10)	.50 (.04)	.33 (.04)	5
S-I	I	1.29 (.04)	.71 (.11)	.39 (.07)	.37 (.09)	4
	C	1.50 (.05)	.74 (.05)	.39 (.02)	.21 (.04)	5
S-II	I	1.33 (.03)	.85 (.09)	.43 (.03)	.36 (.11)	4
	C	1.37 (.05)	.86 (.06)	.47 (.04)	.31 (.03)	5
PF	I	1.36 (.07)	.83 (.07)	.54 (.07)	.30 (.05)	3
	C	1.38 (.04)	.81 (.03)	.51 (.05)	.31 (.06)	3

[a]See text for methods of calculation.

[b]Variance in the N for each cortical area from that noted in the text is the result of loss of tissue during staining and preparation.

order-interfaces as shown by the significant interaction of Condition and Order-interface ($F = 3.51$, $df = 3/21$, $p < .05$). Post hoc testing with the HSD test showed the effect to be primarily on the 2nd- to 3rd-order interface, with controls having a higher mean ratio than deprived animals. The S-I also showed a significant interaction effect of Rearing x Order-interface ($F = 3.39$, $df = 3/21$, $p < .05$). Although post hoc testing did not show any individual Order-interface significance in S-I, the greatest mean

difference between the 2 groups was at the 1st- to 2nd-order interface, where controls showed a higher mean Br.

Discussion

The findings of this study show that partial social isolation can result in changes in neuroanatomical structure in specific areas of the neocortex. Other areas of the cortex (e.g., visual) are not affected by the restricted rearing, arguing against some sort of general hormonal influence (such as that seen with growth hormone by Clendinnen and Eayrs, 1961) affecting brain development in these animals.

One form of sensory restriction, dark-rearing, has been shown to result in a decrease in branching (Coleman & Riesen, 1968) and modification of branching pattern (Borges & Berry, 1976). However, note that Borges and Berry (1976) exclusively examined type 7 cells of Jones (1975) whereas we systematically attempted to exclude

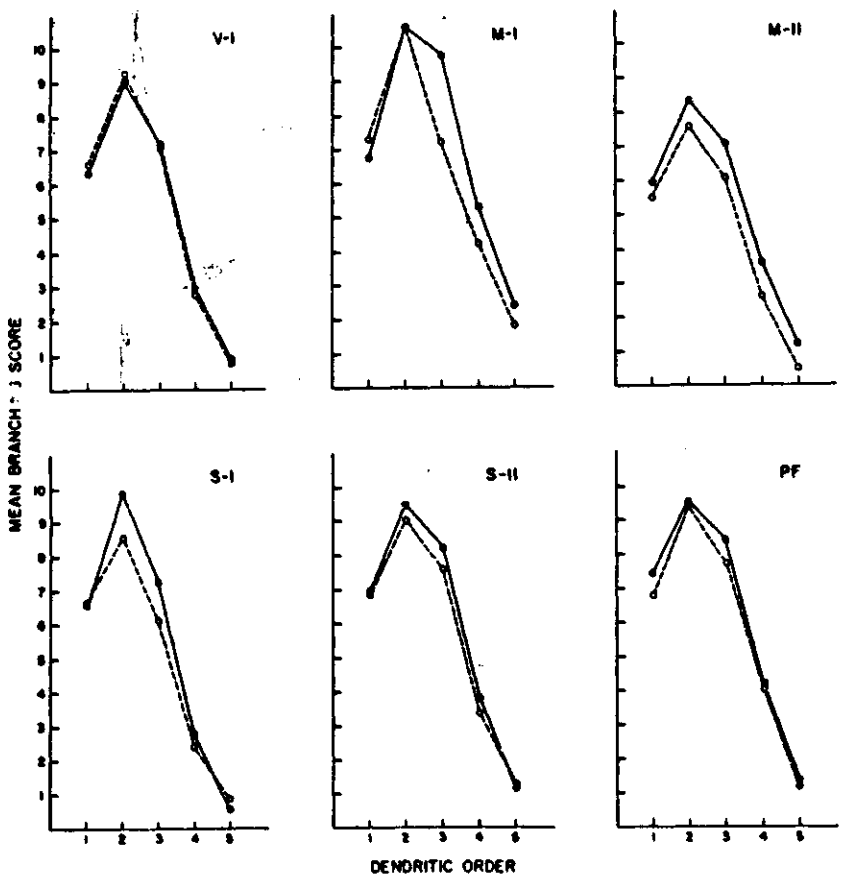

Fig. 2. Mean numbers of dendritic branches for orders 1-5 of Layer IV stellate cells for the 6 cortical areas. Solid circles and lines represent control animals; open circles and broken lines denote the deprived group.

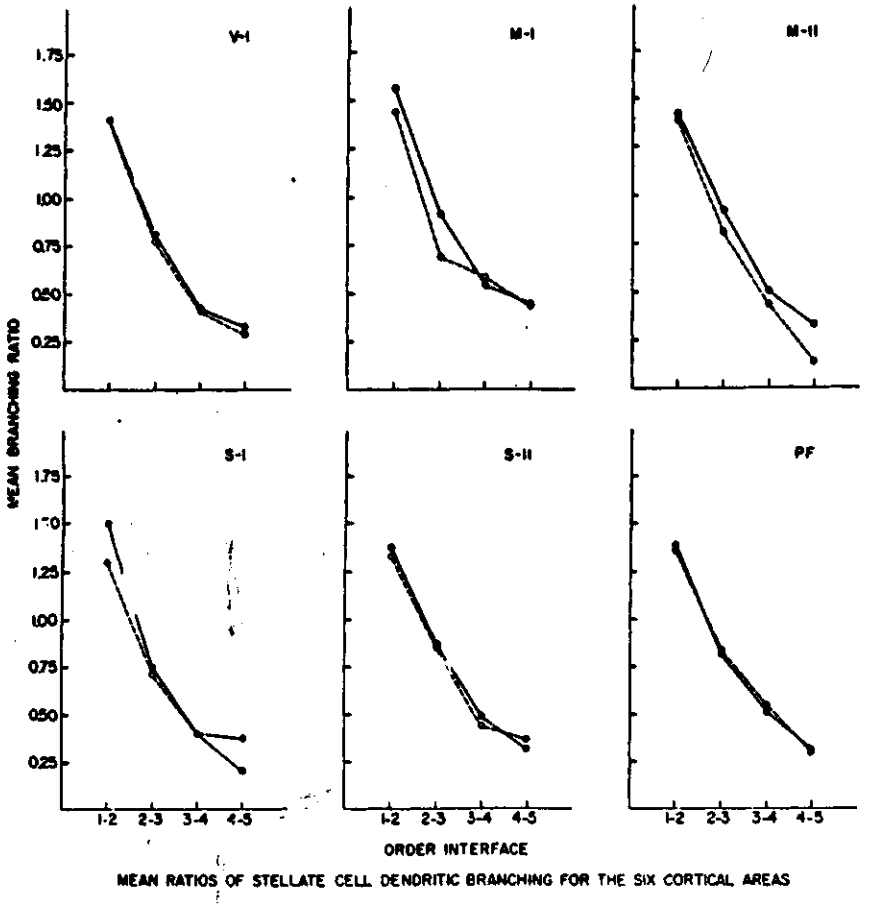

Fig. 3. Mec . branching ratios at each order interface for the 6 cortical areas. Code same as for Fig. 2.

this type of cell in our study. We suggest that the decreased rate of branching in S-I is a result of somatosensory deprivation supporting Prescott's (1970) contention of somatosensory deprivation being part of the social isolation paradigm.

The decreased branching in M-I cortex of the socially deprived animals may be explained by several, but not mutually exclusive, hypotheses: Restriction of somatosensory input may result in less input to M-I effecting a functional deafferentation through decreased input. (This first explanation is weak in that the effects in M-I were much larger than those in S-I.) Alternatively, the small size of the deprivation cage may restrict movement and hence restrict dendritic development, or stereotypic behavior may exclude other behaviors and thereby restrict dendritic growth. Another tenable hypothesis is that the deprived rearing environment removes some salient aspect of the normal environment necessary for development of M-I cortex. This final explanation seems reasonable in light of the findings by Mason and Berkson (1975) that movement of the surrogate can minimize some behavioral abnormalities seen during social isolation. Studies presently in progress in this laboratory are attempting to support or reject these hypotheses.

Notes

This research was supported by Grants EY 00573 and HD 10408 from the National Institutes of Health, USPHS.

References

Borges, S., and Berry, M. (1976). Preferential orientation of stellate cell dendrites in the visual cortex of the dark-reared rat. *Brain Res., 112*:141-147.

Clendinnen, B. G., and Eayrs, J. T. (1961). The anatomical and physiological effects of prenatally administered somatotrophin on cerebral development in rats. *J. Endocrinol., 22*:183-193.

Coleman, P. D., and Riesen, A. H. (1968). Environmental effects on cortical dendritic fields. I. Rearing in the dark. *J. Anat., 102*:363-374.

Eastman, R. F., and Mason, W. A. (1975). Looking behavior in monkeys raised with mobile and stationary artificial mothers. *Dev. Psychobiol., 8*:213-221.

Jones, E. G. (1975). Varieties and distribution of non-pyramidal cells in the somatic sensory cortex of the squirrel monkey. *J. Comp. Neurol., 160*:205-268.

Kirk, R. E. (1968). *Experimental Design: Procedures for the Behavioral Sciences.* Belmont: Brooks/Cole

Mason, W. A., and Berkson, G. (1975). Effects of maternal mobility on the development of rocking and other behavior in rhesus monkeys: A study with artificial mothers. *Dev. Psychobiol. 8*:197-211.

Prescott, J. W. (1970). Early somatosensory deprivation as an ontogenetic process in the abnormal development of the brain and behavior. In J. E. Goldsmith and J. Moor-Jankowski (Eds.), *Medical Primatology*. Basel: Karger. Pp. 356-375.

Ramon-Moliner, E. (1970). The Golgi-Cox technique. In J. H. Nauta and S. O. E. Ebbeson (Eds.), *Contemporary Research Methods in Neuroanatomy*. New York: Springer-Verlag. Pp. 32-55.

Suomi, S. J., Harlow, H. F., and Kimball, S. D. (1971). Behavioral effects of prolonged partial social isolation in the rhesus monkey. *Psychol. Rep., 29*:1171-1177.

Winer, B. J. (1962). *Statistical Principles in Experimental Design.* New York: McGraw-Hill.

Woolsey, C. N. (1958). Organization of somatic sensory and motor areas in the cerebral cortex. In H. F. Harlow and C. N. Woolsey (Eds.), *Biological and Biochemical Bases of Behavior.* Madison: University of Wisconsin Press. Pp. 63-82.