

**EXPERIENTIAL STIMULATION AS A TREATMENT FOR EARLY BRAIN
DAMAGE**

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BSc, University of Lethbridge, 1977

A Thesis
Submitted to the Council on Graduate Studies
of the University of Lethbridge
in Partial Fulfilment of the
Requirements for the Degree

MASTER OF SCIENCE

LETHBRIDGE, ALBERTA
March, 2001

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DEDICATION

In memory of Daniel Ross Valgardson (March 7, 1961 - October 30, 1992)

THESIS ABSTRACT

The current work explores the therapeutic potential of experiential treatments for enhancing functional recovery and anatomical change after early brain damage. Normal rats and rats with perinatal cortical lesions (P2 or P7) were exposed to one of the following treatments: complex housing as juveniles, complex housing as adults, prenatal tactile stimulation, postnatal tactile stimulation, or postnatal handling (removal from the nest with no additional stimulation). Behavior was assessed in adulthood using the Morris water task and the Whishaw reaching task. There were sex differences in the details of the effect of experience on both behavioral recovery and brain morphology. For both sexes treatments initiated prior to or immediately after brain injury were most effective in improving functional outcome. This was correlated with changes in dendritic arborization and Acetylcholinesterase staining. The results suggest that behavioral treatments can be used to stimulate functional recovery after early brain injury.

ACKNOWLEDGEMENT

The author would like to acknowledge Grazyna Gorny, Reed Kindt and Dawn Danka for their technical expertise and help with various aspects of this work; Dr. Bryan Kolb for providing a nurturing and stimulating graduate student environment; Dr. Ian Wishaw for his interesting and challenging course work; Dr. Chris Bender, and Dr. Brian Bland for agreeing to serve on my thesis committee; Ernie and Maureen Valgardson and Glen and Dorothy Gibb for their continuing support of my education; Claudia Gonzalez for her friendship and encouragement; Bill, Jeff and Kirsty for allowing me "time off" from household and family duties to pursue this degree and especially Bill for insisting that I do it.

ENVIRONMENTAL STIMULATION AS A TREATMENT FOR EARLY BRAIN DAMAGE

TABLE OF CONTENTS

1. INTRODUCTION	1.
1.1. CORTICAL PLASTICITY	3.
1.1.1. The rat as a model of cortical plasticity	3.
1.1.2. Cortical plasticity: A historical perspective	5.
1.1.3. Aging and cortical plasticity	6.
1.1.4. Brain injury and cortical plasticity	7.
1.1.4.1. Functional and anatomical sequelae of early brain injury	10.
1.1.5. Sex hormones and cortical plasticity	12.
1.1.6. Stress hormones and cortical plasticity	13.
1.2. ENVIRONMENTAL STIMULATION	13.
1.2.1. Environmental enrichment and normal animals	13.
1.2.2. Environmental enrichment and early brain- damaged adults	14.
1.2.3. Environmental enrichment and early brain- damaged weanlings	16.
1.2.4. Why tactile stimulation?	17.
1.3. BEHAVIORAL AND ANATOMICAL ASSESSMENT	19.
1.3.1. Behavioral Tasks	19.
1.3.1.1. Morris water task	19.

1.3.1.2. Whishaw reaching task	20.
1.3.1.3. Circadian activity	21.
1.3.2. Physiological and Anatomical Assessments	22.
1.3.2.1. Urine corticosterone	22.
1.3.2.2. Golgi method	23.
1.3.2.3. Acetylcholinesterase histochemistry	24.
1.4. THESIS CONTENT AND ORGANIZATION	26.
2. EXPERIMENT 1: EXPERIENCE AND THE CHANGING BRAIN	28.
2.1. ABSTRACT	28.
2.2. INTRODUCTION	29.
2.3. MATERIALS AND METHODS	31.
2.3.1.1. Subjects: Male rats	31.
2.3.1.2. Subjects: Female rats	31.
2.3.2. Enrichment procedures	31.
2.3.3. Anatomical methods	34.
2.3.4. Statistical analyses	36.
2.4. RESULTS	36.
2.4.1. Behavioral observations	36.
2.4.2. Anatomical results: Male rats	37.
2.4.2.1. Gross morphology: Brain and body weight	37.
2.4.2.2. Dendritic arborization	40.
2.4.2.3. Spine density	44.
2.4.3. Anatomical results: Female rats	47.

2.4.3.1. Gross morphology: Brain and body weight	48.
2.4.3.2. Dendritic branching	49.
2.4.3.3. Spine density	52.
2.5. DISCUSSION	54.
2.5.1. Experience and the changing brain	54.
2.5.2. Qualitative differences in spine density	55.
2.5.3. Sex and the changing brain	56.
2.5.4. Aging and the changing brain	58.
3. EXPERIMENT 2: EXPERIENCE AND THE INJURED BRAIN	60.
3.1. ABSTRACT	60.
3.2. INTRODUCTION	61.
3.3. MATERIALS AND METHODS	63.
3.3.1. Experiment 2A	63.
3.3.1.1. Subjects	63.
3.3.1.2. Surgical procedures	63.
3.3.1.3. Enrichment procedures	64.
3.3.1.4. Behavioral methods	65.
3.3.1.5. Anatomical methods	66.
3.3.2. Experiment 2B	66.
3.3.2.1. Subjects	66.
3.3.2.2. Enrichment procedures	67.
3.3.2.3. Behavioral methods	68.

3.3.2.4. Anatomical methods	68.
3.3.3. Experiment 2C	70.
3.3.3.1. Subjects	70.
3.3.3.2. Enrichment procedures	70.
3.3.3.3. Behavioral methods	71.
3.3.3.4. Anatomical methods	72.
3.4. BEHAVIORAL RESULTS	73.
3.4.1. General Behavioral Observations	73.
3.4.2. Morris Water Task	74.
3.4.2.1. General observations	74.
3.4.2.2. Experiment 2A	74.
3.4.2.3. Experiment 2B	77.
3.4.2.4. Experiment 2C	80.
3.4.3. Skilled reaching	82.
3.4.3.1. Experiment 2C	82.
3.5. ANATOMICAL RESULTS	84.
3.5.1. General observations	84.
3.5.2. Brain weight and body weight	85.
3.5.3. Dendritic arborization and spine density	88.
3.5.3.1. Experiment 2B	88.
3.5.3.2. Experiment 2C	90.
3.5.3.2.1. Dendritic length	90.
3.5.3.2.2. Spine density	93.

3.6. DISCUSSION	96.
3.6.1. Complex housing increases dendritic arborization	97.
3.6.2. The effects of experience on spine density are age-dependent	98.
3.6.3. The effects of cortical lesions in infancy are age-dependent	99.
3.6.4. Experience can attenuate the effect of early cortical injury but earlier is better	100.
3.6.5. There may be limits to cortical plasticity after early cortical lesions	101.
3.6.6. The behavioral and anatomical effects of P7 frontal lesions are sexually dimorphic	102.
4. EXPERIMENT 3: TACTILE STIMULATION AFTER CORTICAL INJURY	104.
4.1. ABSTRACT	104.
4.2. INTRODUCTION	105.
4.3. MATERIALS AND METHODS	105.
4.3.1. Subjects	105.
4.3.2. Surgical procedures	106.
4.3.3. Stimulation procedure	107.
4.3.4. Behavioral tasks	108.
4.3.4.1. Morris water task	108.
4.3.4.2. Skilled reaching	109.
4.3.5. Anatomical Methods	110.
4.4. BEHAVIORAL RESULTS	112.

4.4.1. Morris water task	112.
4.4.2. Skilled reaching	115.
4.5. ANATOMICAL RESULTS	116.
4.5.1. General observations	116.
4.5.2. Brain weight	117.
4.5.3. Dendritic length	118.
4.5.4. Spine density	120.
4.6. DISCUSSION	123.
5. EXPERIMENT 4: PRENATAL VERSUS POSTNATAL TACTILE STIMULATION	126.
5.1. ABSTRACT	126.
5.2. INTRODUCTION	127.
5.3. MATERIALS AND METHODS	129.
5.3.1. Subjects	129.
5.3.2. Surgical procedures	130.
5.3.3. Enrichment procedures	130.
5.3.4. Behavioral Methods	131.
5.3.4.1. Morris water task	131.
5.3.4.2. Skilled reaching	132.
5.3.4.3. Circadian activity	133.
5.3.5. Anatomical Methods	134.
5.3.5.1. Histological procedures	134.
5.3.5.2. Anatomical analyses	135.

5.4. BEHAVIORAL RESULTS	136.
5.4.1. Morris water task	136.
5.4.2. Skilled reaching	139.
5.4.3. Circadian activity	142.
5.4.4. Urine basal corticosterone levels	145.
5.5. ANATOMICAL RESULTS	147.
5.5.1. Body weight	147.
5.5.2. Brain weight	149.
5.5.3. Cortical thickness	151.
5.5.4. Lesion size	153.
5.5.5. Acetylcholinesterase Density	155.
5.6. DISCUSSION	158.
5.6.1. Prenatal stroking improves functional recovery	160.
5.6.2. Prenatal stroking and postnatal handling have different effects on males and females	160.
5.6.3. Postnatal handling does not improve functional recovery after early brain damage	162.
5.6.4. Prenatal and postnatal experience alters basal corticosterone levels	164.
5.6.5. Stroking and changes in acetylcholinesterase levels	165.
6. GENERAL DISCUSSION	166.
6.1 Novel Findings	168.
6.1.1. Experiential treatments stimulate cortical plasticity and functional recovery	169.
6.1.2. The age of the subject at the time of treatment	

plays a major role in resulting plasticity and/or recovery.	175.
6.1.3. There are sex differences in response to experiential stimulation that may depend on age at lesion and age at treatment.	176.
6.1.4. Damaged brains respond differently than normal brains to environmental treatment.	178.
6.1.5. Early environmental intervention alters HPA responsivity and may ultimately have an impact,	179.
6.1.6. Some behavioral deficits that result from early brain are more resistant to remediation than are others.	181.
6.2. Proposed mechanisms of experiential treatment as therapy for early brain damage	182.
6.2.1. Mechanisms that may mediate the <i>complex housing</i> treatment effect.	182.
6.2.2. Mechanisms that may mediate the <i>prenatal</i> tactile stimulation treatment effect.	185.
6.2.3. Mechanisms that may mediate the <i>postnatal</i> tactile stimulation treatment effect.	187.
6.3. Conclusion	188.
6.4. Future Directions	191.
7. REFERENCES	194.

LIST OF TABLES

Table 1.1.	Effects of frontal cortical injury at various ages	10.
Table 2.1.	Summary of brain weights for the males rats	38.
Table 2.2.	Summary of the body weights for the male rats	39.
Table 2.3.	Summary of dendritic length in Par 1: males	42.
Table 2.4.	Summary of dendritic length in Occ1: males	43.
Table 2.5.	Summary of spine density in Par1: males	46.
Table 2.6.	Summary of spine density in Occ1: males	46.
Table 2.7.	Summary of brain weights for females	48.
Table 2.8.	Summary of body weights for the females	49.
Table 2.9.	Summary of dendritic length in Par1: females	51.
Table 2.10.	Summary of dendritic length in Occ1: females	51.
Table 2.11.	Summary of spine density in Par1: females	53.
Table 2.12.	Summary of spine density in Occ1: females	53.
Table 3.1.	Summary of brain weights for rats in Exp 2A	85.
Table 3.2.	Summary of body weights for rats in Exp 2A	86.
Table 3.3.	Summary of brain weights for males: Exp 2B, 2C	86.
Table 4.1.	Summary of brain weight	118.
Table 5.1.	Summary of body weights for females	149.
Table 5.2.	Summary of body weights for males	149.
Table 5.3.	Summary of brain weights for females	150.
Table 5.4.	Summary of brain weights for males	151.
Table 5.5.	Summary of cortical thickness: females	152.

Table 5.6.	Summary of cortical thickness: males	153.
Table 5.7.	Relative density of AchE stain	157.

LIST OF FIGURES

Figure 1.1.	Cortical Plasticity	7.
Figure 1.2.	Condominium	15.
Figure 1.3.	Postnatal tactile stimulation	18.
Figure 1.4.	Prenatal tactile stimulation	19.
Figure 1.5.	Morris water maze	20.
Figure 1.6.	Whishaw reaching boxes	21.
Figure 1.7.	Circadian activity monitoring system	22.
Figure 1.8.	Golgi Stain	24.
Figure 1.9.	Acetylcholinesterase stain	26.
Figure 2.1.	Condominium	33.
Figure 2.2.	Drawings of neurons	40.
Figure 2.3.	Graph of dendrites in Par1: males	41.
Figure 2.4.	Graph of spine density in males	44. 45.
Figure 2.5.	Graph of dendrites in Par1: females	50.
Figure 3.1.	Water maze performance: heading angles	76.
Figure 3.2.	Water maze performance: sum latency	77.
Figure 3.3.	Water maze acquisition: adult enriched	78.
Figure 3.4.	Water maze sum latency: adult enriched	79.
Figure 3.5.	Water maze latency: P7 operates	82.
Figure 3.6.	Reaching for food: P7 operates	83.
Figure 3.7.	Typical lesion from a P2 frontal	84

Figure 3.8.	Summary of apical branching	89.
Figure 3.9.	Summary of spine density	90.
Figure 3.10.	Summary of apical branches	91.
Figure 3.11.	Summary of branch number	92.
Figure 3.12.	Summary of spine density: Par1	95.
Figure 3.13.	Summary of spine density: Occl	96.
Figure 4.1.	Water maze performance: Frontals	114.
Figure 4.2.	Water maze performance: Parietals	115.
Figure 4.3.	Skilled reaching: Frontals	116.
Figure 4.4.	Summary of dendritic length: apical	119.
Figure 4.5.	Summary of dendritic length: basilar	120.
Figure 4.6.	Summary of spine density: apical	121.
Figure 4.7.	Summary of spine density: basilar	122.
Figure 5.1.	Water maze performance: acquisition	138.
Figure 5.2.	Water maze performance: sum latency	139.
Figure 5.3.	Reaching performance of females	141.
Figure 5.4.	Reaching performance of males	142.
Figure 5.5.	Circadian activity: females	144.
Figure 5.6.	Circadian activity: males	145.
Figure 5.7.	Glucocorticoid Levels	147.
Figure 5.8.	Lesion size	154.
Figure 5.9.	Lesion cavity size	155.

Figure 5.10.	Summary of treatment effects	159.
Figure 5.11.	Effect of sex hormones on cortical plasticity	161.
Figure 6.1.	Recovery potential after brain damage	179.
Figure 6.2.	Summary of effectiveness of experience	190.

LIST OF ABBREVIATIONS

AchE	Acetylcholinesterase
ANOVA	Analysis of variance
BDNF	Brain derived neurotrophic factor
bFGF	Basic fibroblast growth factor
B IA	Brain Injury Association of Alaska
Cg	Cingulate cortex
CICH	Canadian Institute of Child Health
Condo	Condominium
CORT	Corticosterone
E	Embryonic day
F	Female
Fr	Frontal cortex
IGF-1	Insulin like growth factor
LSD	Least significant difference
M	Male
NA	Not available
NGF	Nerve growth factor
NMDA	N-methyl-D-aspartate
NMDAR	N-methyl-D-aspartate receptor
OC2MM	Occipital cortex (2) medial
OC2ML	Occipital cortex (2) lateral
Occ1	Occipital cortex
P	Postnatal day
PL	Prelimbic cortex
Par 1	Parietal cortex
PPC	Posterior parietal cortex
REM	Rapid eye movement
SEM	Standard error of the mean

EXPERIENTIAL STIMULATION AS A TREATMENT FOR EARLY BRAIN DAMAGE

1. INTRODUCTION

Although brain injury is most commonly observed in males aged 15 to 24, or people over 75 (Brain Injury Association of Alaska [BIA of Alaska], 2000), there is a significant risk of brain trauma during gestation and infancy. Low birth weight (under 2500 grams) is associated with perinatal illness and a higher rate of long-term cognitive problems such as cerebral palsy and learning difficulties. In Canada approximately 6% of full-term infants are classified as low birth weight. The rate of preterm birth is rising in Canada: 6.1% in 1985 to 7.1% in 1997 (Canadian Institute of Child Health [CICH], 2000). Although not all preterm births are low birth weight, early preterm birth is “especially associated with perinatal illness, neonatal death, and long-term complications” (CICH, 2000). These figures reflect prenatal factors that affect infant health and vitality but do not include statistics that detail birth trauma or anoxia at parturition and postnatal brain injury.

In the U.S.A., six in every ten thousand children from birth to age fourteen sustain brain injury annually (BIA of Alaska, 2000). Information from the same source notes that “injuries to the brain are among those most likely to result in irreversible damage.” The financial impact of health care and rehabilitation for children suffering from postnatal and childhood brain injury exceeds forty eight billion dollars per year in America. These costs

highlight a need to implement research designed to determine ways to maximize recovery from early brain injury.

Brain plasticity is a term used to describe structural changes in the brain. Brain plasticity could reflect addition of new neural connections with loss or modification of existing connections and these changes arise primarily under four conditions: 1) developmental plasticity- associated with the immature brain as it begins to acquire sensory processing abilities; 2) activity-dependent plasticity- associated with changes in the body that alter sensory processing in the brain (i.e. visual problems in young children or drug addiction); 3) plasticity of learning and memory- resulting from altered behavior based on new sensory information; 4) injury-induced plasticity- associated with changes in the brain resulting from injury (John F. Kennedy Center for Research on Human Development, Vanderbilt University, 2000). It is assumed that the same general mechanisms underlie all forms of brain plasticity.

It is easy to suppose that some parts of the brain are likely to possess a greater potential for plasticity than others. In particular, the mammalian cortex has many attributes that make it an interesting place to look for a correlation between anatomical flexibility and alterations in behavior. Regional variation in cortical morphology specifies the functional organization of cerebral cortex. Area- specific differences in architecture include variation in cell size and density, distribution of neurotransmitters and their receptors, and differences in afferent and efferent connections. The

functional specificity of the cerebral cortex thus makes it possible to investigate the consequences of experience. For example, visual stimulation should have anatomical consequences in occipital cortex which do not generalize to cortical areas that are not involved in visual function. Another feature of the architecture of the cortex that makes it ideal for studying mechanisms controlling plasticity is the intrinsic cortical circuit. The intrinsic cortical circuit is a local connection formed by neighboring pyramidal neurons and likely represents the type of connection that is most easily altered. Neurons make extensive arborizations (both dendritic and axonal) to connect with nearby cells and these connections account for approximately 70% of the excitatory input on a layer II/III pyramidal cell (Nicoll & Blakemore, 1993). Thus, it appears that local connections represent most of the output of pyramidal neurons and these connections provide an ideal substrate for mechanisms of plasticity. By gaining an understanding of how to initiate effective alterations in connectivity through treatments or interventions it may be possible to develop strategies that will improve functional recovery after brain damage.

1. 1. CORTICAL PLASTICITY

1. 1. 1. The rat as a model of cortical plasticity

The value of rats as experimental subjects for studying cortical function has often been challenged (usually by scientists who study lower primates such as macaque monkeys). Undoubtedly, one of the biggest advantages of

using rats as subjects for neuroscientific research is cost-effectiveness. Not only are rats cheaper to buy, they are much cheaper to maintain. There are added advantages such as a relatively short gestational period and quick maturation that make rats ideal for studies on development and aging. It is also possible to use many animals in a study to reduce the effects of individual variation.

Rat cortical structure is much simpler than that of primates and as such should be easier to understand. It is relatively easy to make consistent, restricted lesions of rat cortex and thus facilitate the study of the functions of particular areas in the brain. (One of the major problems in studies of human brain damage is the difficulty in finding subjects with equivalent lesions in extent, location, and age at injury.)

It is assumed that the brain controls behavior. As a result, changes in brain structure should translate into behavioral change (and vice versa). A neuropsychology battery of tests for assessment of behavior in the rat has been described in detail by Whishaw, Kolb and Sutherland (1983). These tests allow the determination of the nature of behavioral deficits following brain injury. Careful behavioral study also enables one to determine if recovery (either partial or complete) has occurred. Clearly, not all aspects of human cortical function can be revealed with rat models, but there are many basic mechanisms of cortical function (including plasticity) that can be determined.

1. 1. 2. Cortical plasticity: A historical perspective

The notion that the structure of the nervous system can be modified by sensory experience is not new. Santiago Ramon y Cajal (1894) proposed that learning could produce long lasting modifications in neuronal structure. In addition, Ramon y Cajal was the first to propose dendritic spines as major sites of neural connections (DeFilipe & Jones, 1988). In 1948, Jerzy Konorski introduced the idea that activity could provide a powerful stimulus for changes in neural organization. Donald Hebb, in 1949, furthered these ideas with the hypothesis that activity-dependent changes in neuronal architecture would be expressed primarily at the level of the synapse. Although much research in the past 50 years has provided evidence to support these proposals (e.g., Bland & Cooper, 1969; Diamond, Lindner, & Raymond, 1967; Greenough, Black, & Wallace, 1987; Rosenzweig, 1971) it has become increasingly clear that environmental stimulation can have a more widespread influence on cerebral architecture. Kolb (1999) in his "ecological theory of cortical organization" highlights the need to address changes in neuronal "environment" as well as changes within the neuron when assessing the effects of experience on the nervous system. Glial structure, function, and proliferation, as well as neurotransmitter levels and growth factor availability have been shown to undergo changes as a result of various environmental manipulations. These alterations can induce changes in brain size and cortical thickness.

1.1.3. Aging and cortical plasticity

A fundamental feature of cortical plasticity is that it changes in nature over the course of the lifetime of an individual. Although it is thought that the infant brain is more ductile than the adult brain, there appear to be stages of development that possess greater potential for plasticity than others. Many elements contribute to cortical plasticity including the generation of neurons and glia, and the formation of synaptic space through the addition of dendrites and dendritic spines. These elements peak at different developmental stages and thus relative cortical plasticity should increase and decrease accordingly. Neuronal birth in the rat begins on about embryonic (E) day 12, or E12 and continues to about E21 with birth occurring on E22 (Uylings, Van Eden, Parnavelas, & Kalsbeek, 1990). Cortical plasticity is relatively low during the first postnatal week at which time neuronal migration occurs. The cerebral cortex is most plastic at the developmental stage during which dendritic and synaptic growth is maximal and the generation of astroglia peaks (Figure 1.1). In the rat this occurs following the arrival of the neurons at their destination during the second postnatal week. Adult animals generally show reduced plasticity that declines more dramatically during senescence. Thus the effects of brain injury and environmental stimulation on an animal should also vary with age.

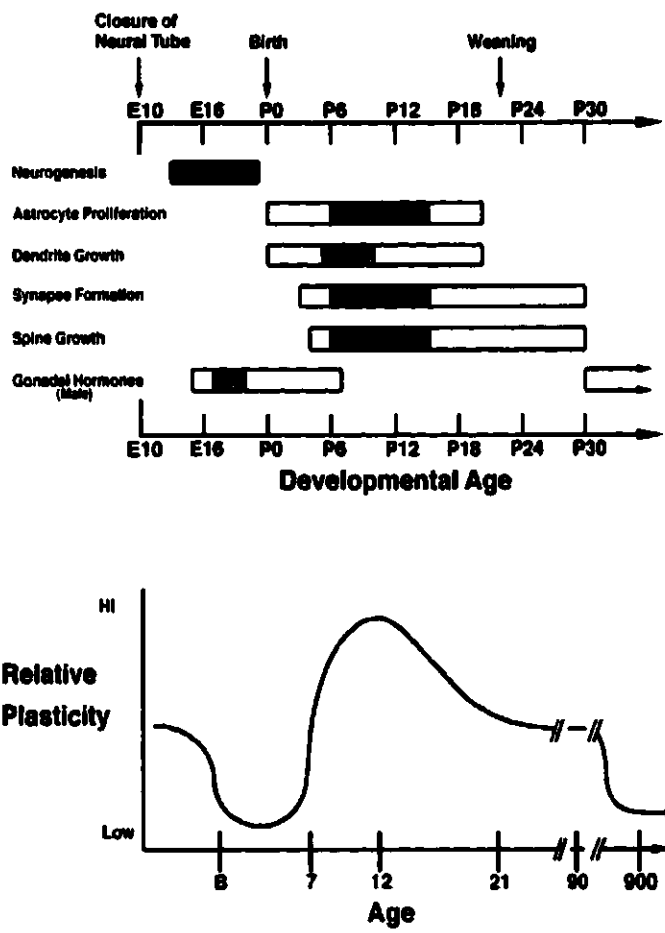


Figure 1.1. Top. Main cellular events related to cortical plasticity. Bars mark the approximate beginning and ending of different processes. The intensity of the shading reflects the intensity of the phenomenon. **Bottom.** Summary of the time-dependent differences in cortical plasticity.

1. 1. 4. Brain injury and cortical plasticity

Systematic study of the effect of early brain damage was begun by Margaret Kennard in the 1930's when she compared unilateral motor cortex lesions in infant and adult monkeys (Kennard, 1938). The impairments in

the infant monkeys were milder than those in the adults, which led Kennard to hypothesize that there had been a change in the cortical organization of the infants. This cortical reorganization was presumed to be supporting the behavioral recovery. In particular, she hypothesized that if some synapses were removed as a consequence of brain injury, "others would be formed in less usual combinations" and that "it is possible that factors which facilitate cortical organization in the normal young are the same by which reorganization is accomplished in the imperfect cortex after injury" (Kennard, 1942, p.239). Although Kennard had much to say regarding the limitations of functional recovery after early brain injury (for a review see Finger & Alml, 1988), it was her demonstration that the consequences of motor cortex lesions in infancy were less severe than similar injury in adulthood that is usually associated with her name, and is commonly referred to as the "Kennard Principle". In the over 50 years since Kennard's experiments, the Kennard Principle has come to reflect the idea that if you are going to have brain damage, have it early because the recovery will be more complete. This does not explain, however, the contrary observations that some kinds of brain damage are actually worse if they are experienced during development. This view was first clearly enunciated by Donald Hebb. In the course of studying children with frontal lobe injuries early in life, Hebb noticed that many children had far more severe functional loss than would be expected from a similar injury in an adult (Hebb, 1947). Hebb concluded that early injuries

may prevent the development of certain intellectual capacities that are critical to normal cognitive development (e.g., Hebb, 1947,1949).

Over the 50 years since Kennard and Hebb's observations, there have been extensive studies on the effects of cortical injury in a variety of laboratory species, especially rats, cats, and monkeys (for a review see Finger & Almli, 1984). Taken together, these studies support the idea that it is the precise developmental age that predicts the functional outcome of early cortical injuries (e.g., Kolb, 1995; Villablanca, Hovda, Jackson, & Infante, 1993).

While undertaking the study of the effects of frontal cortex damage in developing rats, Kolb and others (e.g., Kolb & Nonneman, 1976, 1978; Kolb, Sutherland, & Whishaw, 1983) noticed that rats with lesions at about seven days of age (a developmental age coincidental with high cortical plasticity) showed remarkable behavioral recovery when tested as adults. Rats given lesions earlier in life (postnatal days one through five), a developmental age coincidental with low cortical plasticity, showed a much poorer functional outcome than rats with lesions at postnatal days seven through ten or rats with similar removals in adulthood. Lesions performed before birth (E18) allowed complete restoration of behavioral function in adulthood (Kolb, Cioe, & Muirhead, 1998). Thus the age at which an animal sustains cortical damage is predictive of functional outcome and dependent on the degree of plasticity the developing brain is experiencing at the time (Table 1.1).

Table 1.1. Summary of the effects of frontal cortical injury at different ages

Age at Injury	Result	Reference
E18	Cortex regrows with odd structure Functional recovery	Kolb et al. 1998a
P 1- P 6	Small brain, dendritic atrophy Dismal functional outcome	Kolb and Gibb 1990
P 7-P 12	Dendrite and spine growth Cortical regrowth Functional recovery	Kolb and Gibb 1990 Kolb et. al 1998b
P 120	Dendritic atrophy, then growth Partial return of function	Kolb 1995

Abbreviations: E 18, embryonic day 18; P, postnatal day , number refers to age in days

Another predictor of functional recovery is the nature of the lesion. A characteristic of the lesion that could affect recovery is its size. If the lesion involves an entire functional area, recovery will be more limited than if part of that region remains intact (Kolb, 1995). Some of the anatomical reorganization required to restore lost function probably occurs in the remaining intact tissue.

1. 1. 4. 1. Functional and anatomical sequelae of early brain damage

Although most of the work done (in Dr. Bryan Kolb's lab) on recovery

from early cortical injury in the rat has focused on recovery processes after frontal cortex lesions, there have also been studies on other cortical regions such as motor cortex, posterior parietal cortex (PPC), and occipital cortex. There are differences in the functional outcome of animals sustaining frontal cortex or more posterior lesions but for the most part these differences arise when the animals sustain brain injury during the second postnatal week, when recovery after frontal cortex removal is good. Some of the behavioral improvement observed seems to be dependent on neurogenesis and filling-in of the lesion cavity in the medial frontal area (Kolb, Gibb, Gorny, & Whishaw, 1998). Rats with PPC lesions, for example, do not show the same degree of behavioral recovery after lesions around postnatal day 10 and the lesion cavity shows no evidence of filling-in. The functional consequences of early damage in these two cortical regions is more comparable. Both PPC and frontal cortex removals in the first postnatal week result in extensive anatomical change and a rather poor behavioral outcome. As both the anatomical and functional consequences following removals during the first postnatal week, are similar for these two brain regions the effects of early removal of the frontal cortex will be presented.

Infant rats have very small brains and the anatomical relationship of the brain to the overlying skull sutures is not fixed as it is in adults (Kolb, 1987). Thus, even with the aid of a surgical microscope it is difficult to make precise lesions before about five days of age. In all of the experiments

discussed in this work, the frontal cortex removals were large and included damage to the bordering motor cortex.

Rats with lesions to frontal cortex before seven days of age show a variety of behavioral deficits when tested as adults on tasks such as tongue-extension, grooming, beam walking, and the Morris water task and their performance is worse than the performance of adult frontal lesion animals on such tasks (Kolb, 1987). (These early operated do not show the same degree of recovery as do animals with frontal lesions at 7-10 days of age.)

Morphological consequences of early frontal lesions include: reduction in brain weight, decreased cortical thickness, aberrant cortical connectivity, general atrophy of dendritic arborization, and a drop in spine density across the cortical mantle (Kolb, Gibb, & van der Kooy, 1994). The poor spontaneous recovery of early brain-damaged animals makes them ideal candidates for experiments aimed at assessing the effectiveness of experience on functional recovery.

1. 1. 5. Sex hormones and cortical plasticity

Work by Juraska (1990) has shown that the cerebral cortex of male and Female rats is structurally different (males have thicker, longer, and wider cortices than do females; sexually dimorphic variation occurs in the thickness of cortical layers of particular brain regions) and respond to experience in different ways. By examining visual cortex, Juraska (1984) showed that males demonstrate greater dendritic proliferation in response to enriched housing

than did females. Thus, assessments of environmental effects should not be made without consideration of possible sex differences.

1. 1. 6. Stress hormones and cortical plasticity

Virtually all animals experience stress at some time during their lives. Chronic stress mediates effects on the neuroendocrine system, which ultimately cause changes in neuronal cell morphology (Stewart & Kolb, 1988). Although much of the stress research conducted to date has focused on the hippocampal formation (e.g., Meaney, Aitken, & Sapolsky, 1987; Gould, McEwen, Tanapat, Galea, & Fuchs, 1997), there is evidence to support the idea that neocortical neurons are also susceptible to stress. Indeed, the receptors for corticosterone (glucocorticoid-receptors -Type II) are located throughout the cerebrum and are particularly dense in frontal cortex. Whether or not stress interacts with environmental stimulation to alter brain morphology is not yet clear but it does seem likely (Kolb & Whishaw, 1998).

1. 2. ENVIRONMENTAL STIMULATION

1. 2. 1. Environmental enrichment and normal animals

In the early 1960's Rosenzweig and others (Rosenzweig, 1971) conducted experiments on complex rearing of postweaning animals and showed that by altering an animal's environment the morphological characteristics of its brain were altered. Resulting changes in brain structure were, however, dependent on how the animal interacted with this novel

environment. If interaction was minimal, little change was observed in the animal's behavior and subsequent change noted in brain structure was also limited. If the animal engaged in a new repertoire of behaviors while interacting with the novel environment, many alterations in brain morphology resulted. Brain weight was increased, as was cortical thickness, glial number, neuron size, dendritic branching, and number of synapses per neuron. The idea that environmental enrichment might work as a therapy following cerebral cortex injury in adult rats is based on this work, and the work of others (for a review, see Will & Kelche, 1992).

1. 2. 2. Environmental enrichment and early brain-damaged adults

In the early enrichment experiments conducted in Kolb's laboratory, large pens with sawdust on the floor were used. Inside the pen a half- bale of straw, various lengths of PVC pipe, branches and assorted toys were placed to allow the occupants access to a variety of stimuli. The major drawback with an environment like this was the lack of opportunity for vertical movement. The enriched environments currently used are tall indoor pens (condominiums) constructed of hardware cloth on the front and sides and blue arborite on the back surface. Runways, ramps and boxes form a part of the architecture of these pens and along with the hardware cloth, afford the inhabitants plenty of climbing opportunities. There are also many bridges, ropes and swings that provide additional potential for activity. Within the pens a variety of PVC pipes, empty cardboard boxes, and infant toys are placed

and routinely changed. Objects such as these provide the animals with forms of novel stimulation (Figure 1.2). These condominiums worked well for our early brain-injured adult animals as they were observed to interact with the environmental stimulation they were offered. During the course of the behavioral testing it became apparent that despite some improvement on certain cognitive tests, behavioral recovery was slight and functional impairments were still obvious. It was then decided to provide the "enrichment" intervention closer to the time of damage.

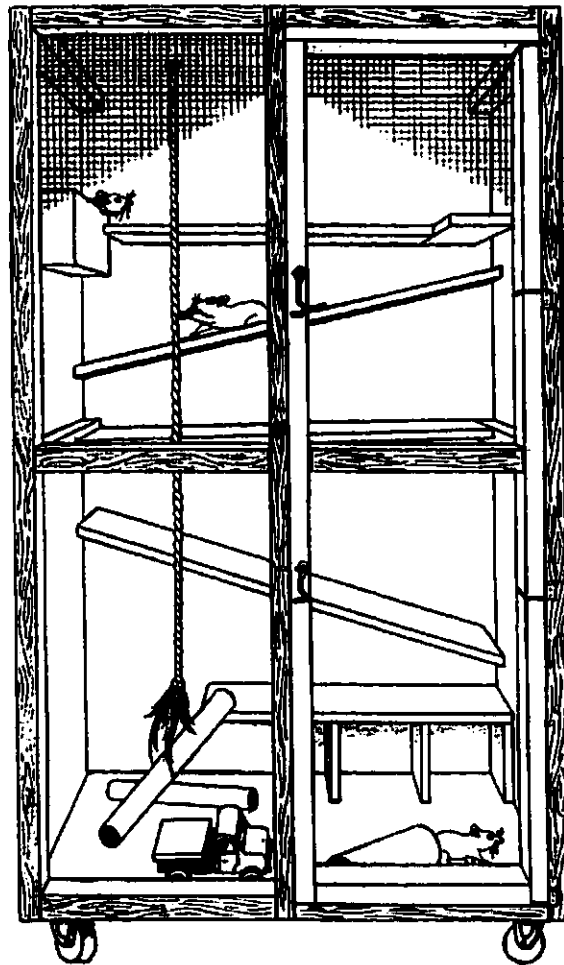


Figure 1.2. A representation of the condominiums used to provide complex housing for environmental stimulation.

1. 2. 3. Environmental enrichment in early brain-damaged weanlings

Weanling rats are at an active stage in their development [characterized by heightened activity levels of behaviors such as jumping, running, and climbing (Bolles & Wood, 1964)] and are thus well suited to life in the condominiums. The motor skills they possess are adequate to allow them access to all levels of the condominiums and they are able to interact with the novel objects, swings, and bridges.

Upon evaluation of the outcome of the enrichment experiments, it was noted (in preliminary studies) that the variation or degree of behavioral recovery found in the lesion animals was related to the age at which the enrichment was offered. In addition, the morphological changes found in the brain following complex housing also varied depending on the age of the animal at the time of enrichment. For the sake of clarity, it was decided that the effects of enrichment at different ages in normal animals (both male and female) should precede any discussion of enrichment effects in lesion animals. Thus, Experiment One describes the findings of how enrichment modifies the brain morphology of normal animals when they are introduced to complex housing as juveniles, young adults or aged adults. Experiment Two describes the effects of enrichment on animals that were given cortical lesions at either postnatal day two (P2) or postnatal day seven (P7). The differences in functional and morphological outcome of animals placed in “enriched housing” as adults (Experiment 2A) or as weanlings (Experiment

2B) is examined. In addition, the effect of early enrichment on early brain-damaged animals that already show good spontaneous functional recovery (Experiment 2C) is explored.

1. 2. 4. Why tactile stimulation?

Although enrichment therapies such as rearing animals in environments full of interactive opportunities, like the condominiums, work well they cannot be used for preweanling animals. In order to determine if environmental interventions were more effective when the treatment was introduced immediately following the lesion, a different method of stimulation had to be employed. Young rats are unable to see and hear until approximately two weeks of age so olfaction and somatosensation comprise the majority of all sensory processing until then. Because tactile stimulation has been shown to stimulate growth in premature infants (Field, Schanberg, Scafildi, et al., 1986) and newborn rats (Schanberg, & Field, 1987), we decided to try tactile stimulation as a treatment for early cortical damage in perinatal rats (Figure 1.3). For the rat pups, stimulation of this sort might be a similar experience to the licking and grooming they receive from their mother.

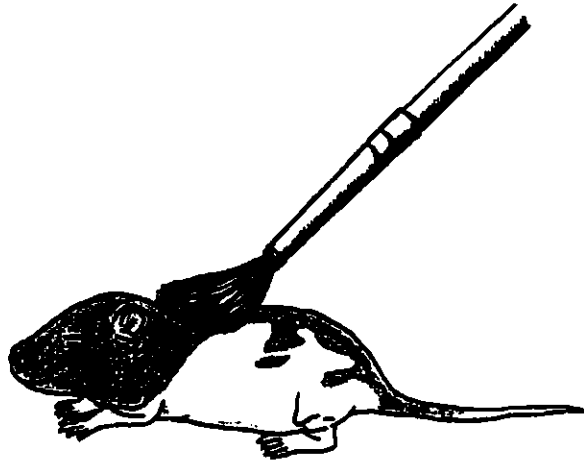


Figure 1.3. Tactile stimulation of an infant rat with a camelhair paintbrush.

Experiment 3 details the effects of post-injury tactile stimulation on behavioral recovery following early damage to either frontal or parietal cortex.

The promising results from the postnatal tactile stimulation experiment led to the idea that prenatal stimulation may also be useful as a therapeutic intervention for early brain damage. Adult female rats were brushed and handled one week before a male was introduced to their home cage (Figure 1.4). Tactile stimulation proceeded through the entire pregnancy and ceased upon the day of parturition. Experiment 4 explores the relative effectiveness of prenatal stimulation as compared to postnatal stimulation in enhancing recovery.

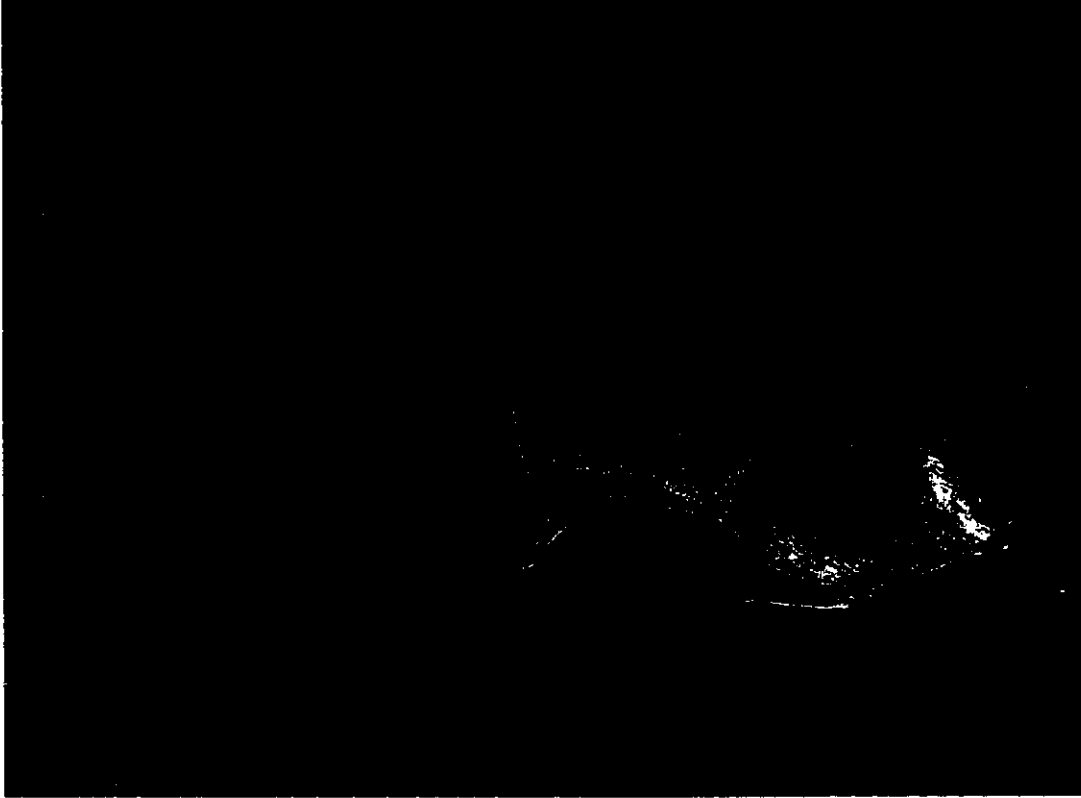


Figure 1.4. Prenatal tactile stimulation

1. 3. BEHAVIORAL AND ANATOMICAL ASSESSMENTS

1. 3. 1. Behavioral Tasks

1. 3. 1. 1. Morris water task

The Morris Water task was developed by Richard Morris (1981) and has been used extensively as a test of learning ability in both normal and brain damaged rats. In one version of this task, rats locate a hidden platform in a

large tank of opaque water by learning the location of static extramaze cues (Figure 1.5). Rats are good swimmers and learn to locate the platform quickly with very little practice. Performance on this task can be assessed by latency to find the platform, heading angle accuracy, and swim distance. Animals with specific cortical lesions show performance deficits on this task and some are entirely unable to learn how to solve it. The observed deficits are not due to motor problems with swimming or standing on the platform but seem more likely due to problems with learning the location of the platform or how to navigate efficiently to it.



Figure 1.5. The Morris Water Maze

1. 3. 1. 2. Whishaw reaching task

Ian Whishaw and his colleagues (e.g., Whishaw, Dringenberg, & Pellis,

1992; Whishaw, Gorny, & Pellis, 1991; Whishaw, O'Connor, & Dunnett, 1986) have developed a procedure to assess the ability of rats to use their forepaws to retrieve food. A rat is trained to reach through metal bars to retrieve chicken feed from a tray at the front of the cage (Figure 1.6). This test is specific for motor skills and performance is measured by the success of the animal to retrieve and ultimately consume food.



Figure 1.6. The Whishaw reaching boxes

1.3.1.3. Circadian activity

Circadian rhythms are the day-night rhythms found in almost all animals. In mammals, sleep-waking behavior and general activity are thought to be controlled by a circadian pacemaker within the suprachiasmatic nucleus of the hypothalamus. Rats are nocturnal animals and as such show a higher level of activity in the dark (Kolb & Whishaw, 2000). Using a computer monitoring system (Figure 1.7) we can assess circadian activity and

determine if there is a shift from the normal species typical behavior following cortical lesions and/or environmental stimulation.

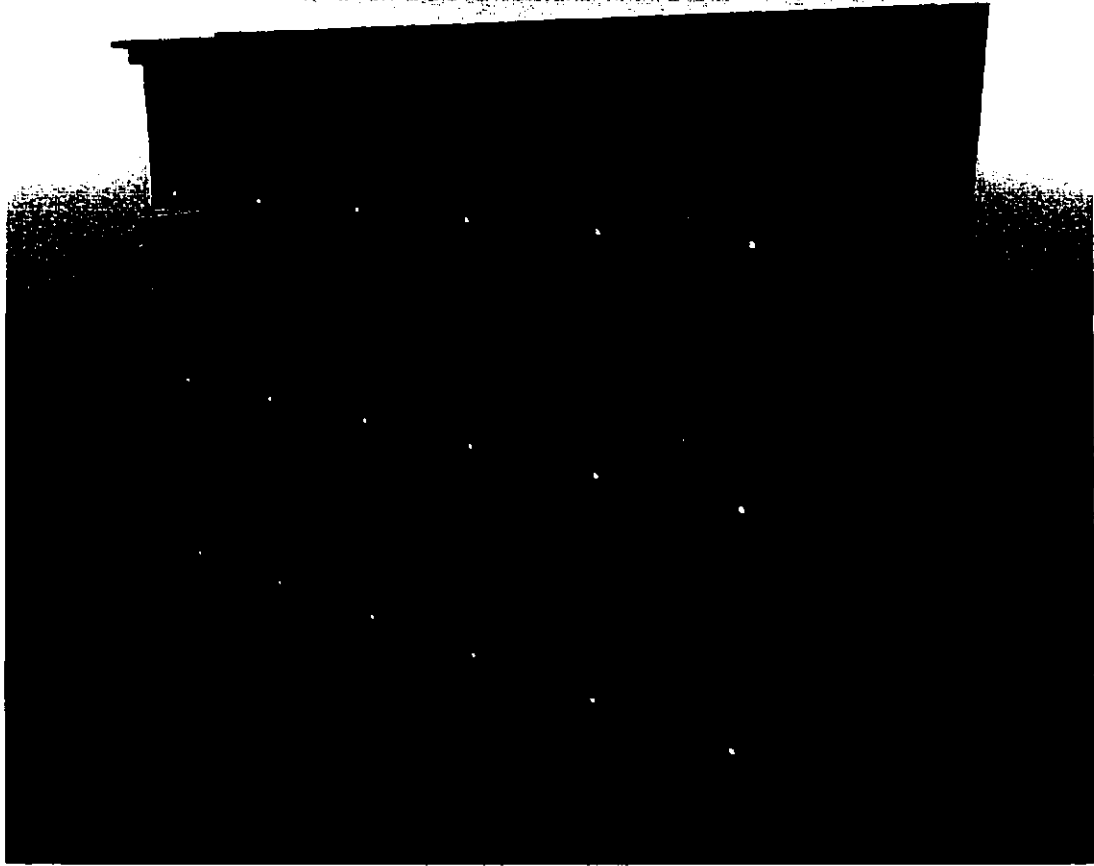


Figure 1.7. Circadian Activity Monitoring System

1. 3. 2. Physiological and Anatomical Assessments

1. 3. 2. 1. Urine corticosterone

Activation of the hypothalamus-pituitary-adrenal (HPA) axis is an adaptive response to stress and is characterized by increased glucocorticoid secretion. There is growing evidence to suggest that stress during prenatal

and postnatal periods of life can alter the adaptive capacities of adult subjects to stress (Levine, Haltmeyer, Karas, & Denenberg, 1967; Liu, Dioro, Tannenbaum, et al., 1997). In a study of the effects of postnatal handling on age-related impairments associated with hippocampal function, Meaney and colleagues (Meaney, Aitken, van Berkel, Bhatnagar, & Sapolsky, 1988b) showed that early postnatal handling of rat pups causes a lower basal level of corticosterone that persists throughout the lifetime of the animal. Assessment of urine corticosterone in adulthood can thus provide evidence for the re-organization of the HPA system following prenatal or postnatal interventions.

1. 3. 2. 2. Golgi method

The Golgi technique was developed in 1873 by Camillo Golgi and was used extensively by early investigators (notably Ramon y Cajal) to define structural features of brain architecture (DeFelipe & Jones, 1988). A major advantage of the Golgi technique is that a small percentage of neurons (1-5%) are randomly stained and these neurons are stained completely (Figure 1.8). As a result it is possible to draw individual neurons and to quantify the amount of dendritic space available, as well as the location and density of dendritic spines. A comparison of neurons from "enriched" animals with neurons from appropriate control animals would thus enable characterization of changes induced by environmental stimulation and in

doing so provide a means of correlating behavioral outcome with brain anatomy.



Figure 1.8. Neuronal morphology as revealed with Golgi-Cox staining

1. 3. 2. 3. Acetylcholinesterase histochemistry

Acetylcholinesterase (AChE) is a catabolic enzyme that is bound to extracellular matrix material found in the synaptic cleft of cholinergic neurons (neurons that secrete acetylcholine). Traditionally, AChE histochemistry has provided a method to study the cholinergic transmitter system in the brain (Figure 1.9). One can assess the normal distribution of AChE and then look for altered distribution or density of staining following experimental intervention such as cortical lesions or environmental stimulation. Although the distribution of AChE is not necessarily correlated with the presence of cholinergic axons, in some systems such as the basal forebrain projection into cortex, it is thought to be a reasonably good marker

(Johnson, 1988). It is clear that the maturation pattern of AchE staining in the cortex occurs at an earlier time than the pattern seen for choline acetyltransferase (an acetylcholine synthesizing enzyme) so the ontogenetic pattern of AchE staining may not mimic the development of cholinergic synapses (Appleyard, 1992). Recent studies examining the function of AchE have revealed that it may play an important role as a neuromodulator with effects on neurite extension and the structural regulation of post-synaptic differentiation (Bravo, Henley, & Rodriguez-Itharralde, 2000). This makes an assessment of AchE staining even more valuable as a tool for studying synaptic plasticity as AchE may play a critical role in this process.

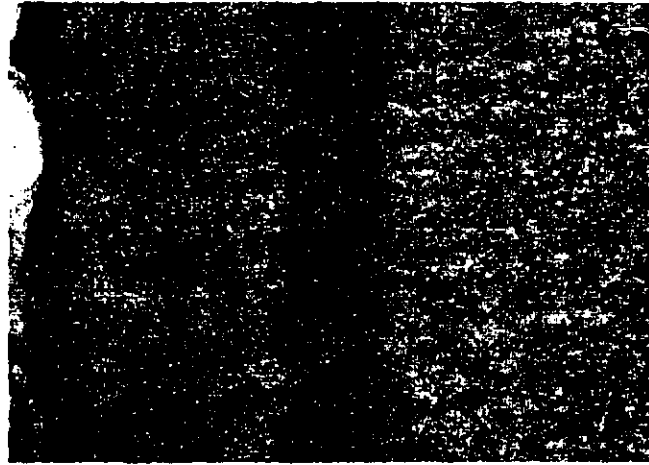
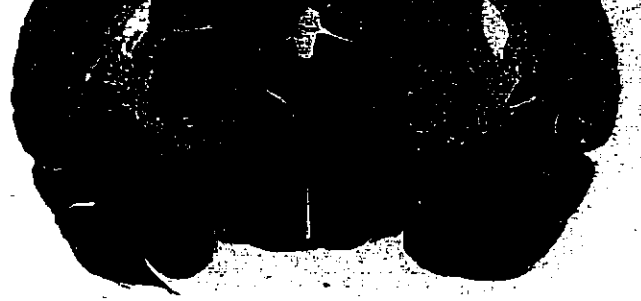


Figure 1.9. Top. Acetylcholinesterase staining of a whole brain section
Bottom. Magnified view of staining in Cingulate Cortex.

1.4. THESIS CONTENT AND ORGANIZATION

This thesis contains four experiments that examine the effect of environment on functional recovery and brain anatomy in rats. Experiment 1 details how the outcome of complex rearing on the neuroanatomy of

normal animals varies depending on the age at treatment and the sex of the subject. Experiment 2 summarizes the results of three experiments (Experiments 2A, 2B, 2C) which examine how experience affects recovery after frontal cortex lesions at two (Experiments 2A, 2B) and seven (Experiments 2C) days of age. The manner in which age at treatment and sex of the subject interact with experience and recovery is considered. Experiment 3 reviews the potential of very early intervention, in the form of tactile stimulation, on recovery after early cortical damage. This experiment studies behavioral and anatomical sequelae of tactile stimulation following both frontal and PPC lesions on postnatal day four. Experiment 4 studies the relative effectiveness of prenatal versus postnatal experience on improving functional recovery in rats after early frontal cortex injury. Taken together, these experiments provide an overview of how functional recovery can be affected by age at injury, age at environmental intervention, and sex of subject following perinatal brain injury.

2. EXPERIMENT 1: EXPERIENCE AND THE CHANGING BRAIN

2.1. ABSTRACT

Male and female rats were placed in the enriched environments for 3 mo either at weaning (22 days) or in young adulthood (120 days). In addition, male rats were given enriched experience in senescence (24 mo). The animals' brains were compared to those of littermate controls who were housed in standard laboratory caging throughout their lifetimes. Relative to the cage-housed animals, enriched male animals at all ages had heavier brains and increased dendritic arborization in both parietal and visual cortical layer III pyramidal neurons. Females showed little increase in dendritic arborization in the youngest animals but significant increases in adulthood. Enrichment had qualitatively different effects on spine density at different ages in both sexes. For males, spine density was increased in the enriched adult and aged rats whereas spine density decreased in the juvenile rats. For females, there was no increase in spine density in the enriched adult rats but there was a decrease in the juvenile rats. There are thus qualitative differences in the effects of experience on cortical morphology that vary with age and sex.

2. 2. INTRODUCTION

The idea that experience modifies brain morphology can be traced back at least to Ramon y Cajal (e.g., 1928) but it was Hebb who made this a central idea in his neuropsychological theory (e.g., Hebb, 1949). Hebb can probably be credited with the first experiment on the behavioral consequences of enriched rearing (Hebb, 1947), but it was not until the group at Berkeley began to demonstrate changes in brain weight, cortical thickness, acetylcholine levels, and dendritic structure that there was any evidence of a structural change induced by experience (e.g., Rosenzweig et al., 1962; 1978). Later, beginning in the 1970s, and continuing still, various groups have focused upon the behavioral and anatomical effects of rearing animals in enriched or deprived environments (for reviews see Greenough, Black & Wallace, 1987; Greenough & Chang, 1988; Juraska, 1990; Kolb & Whishaw, 1998; Walsh, 1982).

In the mid 1980s we began a series of experiments designed to look at the effect of enriched experience on recovery from brain damage sustained at different ages (e.g., Kolb & Elliott, 1987; Kolb & Gibb, 1991). In the course of these experiments it became obvious that experience had effects on the uninjured brain that varied both with age at the time of experience as well as the details of the experience and the sex of the animal. Furthermore, these factors each interacted with the effects of cortical injury to make a very complex story. We therefore have spent nearly a decade parceling out the various effects and trying to make sense of them. This paper begins a series of

papers that are designed to present a comprehensive, and hopefully straightforward, summary of our conclusions. The current paper examines the effects of age and sex. Previous studies have suggested that the male and female cortex may respond to the environment differently (e.g., Juraska, 1990), but these studies were performed with juvenile animals. Previous studies have also emphasized that both young and aged rats show changes in response to enriched housing (e.g., Black, Greenough, Anderson, & Isaacs, 1987), but none of these studies examined spine density. We therefore manipulated sex and age and then measured dendritic arborization and spine density in layer III cortical pyramidal neurons.

In the current series of experiments, rats were exposed to special environments either as juveniles (postnatal days 22-120), young adults (day 120-220), or in senescence (24-27 mo). (Only male rats were studied in senescence.) Littermate control animals were group-housed in standard hanging laboratory cages during the same periods. The brains of the animals were all prepared for Golgi-Cox staining and subsequent analysis of dendritic arborization and spine density. In order to facilitate description of the results, we first consider the effect of age in juvenile, young adult, and senescent male rats and then consider the effect of sex in the juvenile and young adult female animals. Finally, we make a direct male-female comparison for juvenile and young animals.

2. 3. MATERIALS AND METHODS

2. 3. 1. 1. Subjects: Male rats

The study was done with 44 male Long-Evans rats derived from Charles River strains, which were divided into three ages (juvenile, n=10; young adult, n=12; old, n=22). The rats in each age group were assigned to either the lab or enriched housing such that body weight was approximately equal in the lab and enriched groups and that approximately equal numbers of animals in the treatment groups came from the same litter.

2. 3. 1. 2. Subjects: Female rats

The study included 22 Long-Evans female rats derived from Charles River strains, which were divided into two ages (juvenile, n=10; young adult, n=12). The animals were assigned to treatment groups using the same criteria as was used for the males. The female rats came from the same litters as the juvenile and young adult male rats.

2. 3. 2. Enrichment procedures

The rats were reared with their mothers in 22 X 44 X 18 cm Plexiglas cages with corn cob chip bedding until they were 22 days of age. The animals were housed in 65 X 26 X 18 cm stainless steel hanging cages (3-4 per cage) in a busy animal facility containing about 300 other rats. The enriched housing

took place in large indoor pens (condominiums¹) measuring 63 X 148 X 187 cm. Three of the walls (sides and front) were made of hardware cloth. The back wall was made of plywood covered with blue arborite, as was the ceiling and floor (Figure 2.1). Two stainless steel cages (22 X 26 X 18 cm) were attached to the upper part of the front wall and another was placed on its side on the cage floor. There also were runways attached to the back wall, which allowed animals to navigate from the floor to a shelf near the top without having to run up the hardware cloth walls. There were two similar condominiums with 4-6 animals housed in each. The condominium floor was covered with about 10 cm of sawdust bedding. The pen was filled with various objects such as lengths of PVC pipe that the animals could run through, tree branches, boxes, discarded children's toys, and other laboratory 'junk'. The objects were changed weekly. The animals were left undisturbed except for daily feeding and weekly cleaning of the condominium.

¹ We have called the pens condominiums to emphasize the vertical aspect of the enriched housing. This differs from the pens used in our earlier studies and those used by most investigators.

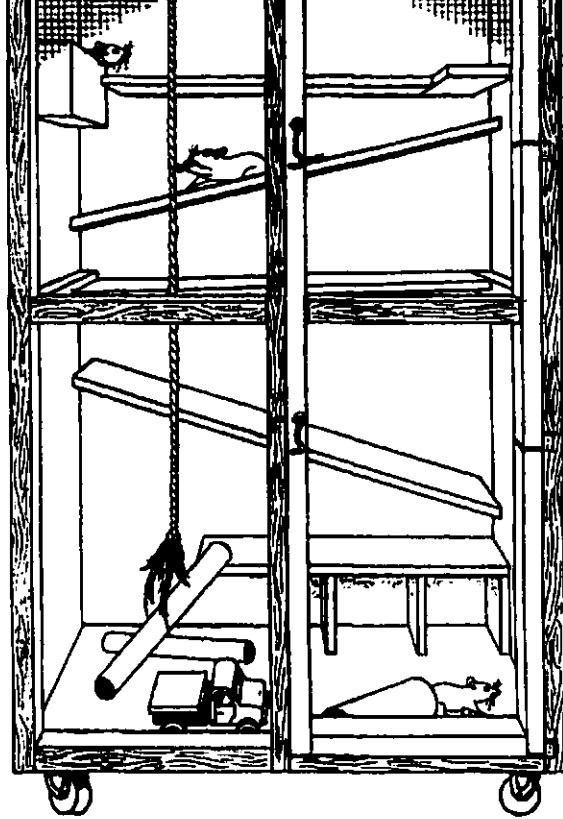


Figure 2.1. Schematic illustration of the rat condominiums. Animals were group housed in these enclosures for three months. The arrangement of the objects in the condominiums was changed semiweekly.

All animals were maintained on a 12:12 hr light/dark cycle. They were given ad lib access to food and water throughout the experiment. Each group of enriched rats was housed in the condominiums for about 95 days, at which time they were removed and their brains were prepared for histological analysis.

2. 3. 3. Anatomical Methods

Following the conclusion of the complex housing the animals were given an overdose of sodium pentobarbital and intracardially perfused with 0.9% saline. The brains were removed and weighed before being immersed whole in 20 ml of Golgi-Cox solution. The brains were left in the solution for 14 days before being placed in a 30% sucrose solution for two to five days, cut on a Vibratome™ at 200 μm , and developed using a procedure described by Gibb and Kolb (1998). Layer II/III pyramidal cells in area Par 1 (Zilles, 1985) were traced using a camera lucida at 250X. In order to be included in the data analysis, the dendritic trees of pyramidal cells had to fulfill the following criteria: (a) the cell had to be well impregnated and not obscured with blood vessels, astrocytes, or heavy clusters of dendrites from other cells; (b) the apical and basilar arborizations had to appear to be largely intact and visible in the plane of section. The cells were drawn and analyzed using two different procedures. In the first, each branch segment was counted and summarized by branch order using the procedure of Coleman and Riesen (1969). Branch order was determined for the basilar dendrites such that branches originating at the cell body were first order; after one bifurcation, second order; and so on. Branch order was determined for the apical dendrites, such that branches originating from the primary apical dendrite were first order and so on. In the second, a Sholl analysis (Sholl, 1956) of ring intersections was used to estimate dendritic length. The number of

intersections of dendrites with a series of concentric spheres at 25 μm intervals from the center of the cell body was counted for each cell. Total dendritic length (in μm) can be estimated by multiplying the number of intersections by 25X.

Cells were chosen by locating the parietal cortex (Par 1, Zilles, 1985) at the level of the anterior commissure and then by drawing each cell in the section that met the criteria listed above. This region is distinct in Golgi-stained sections and normally it is possible to obtain sufficient cells from two adjacent sections. Similarly, cells were chosen in the occipital cortex (Occ 1, Zilles, 1985). Ten cells were drawn in each hemisphere of each rat. The statistical analyses were done by taking the mean of the measurements on the ten cells for each hemisphere of each subject.

Spine density was measured from one apical dendritic branch in the terminal tuft, one secondary apical branch beginning about 50% of the distance between the cell body and terminal tuft, one basilar terminal branch, which was always a fourth order terminal branch, and one secondary basilar branch. Spine density measures were made from a segment greater than 10 μm in length, and usually about 50 μm . The dendrite was traced (1000X) using a camera lucida drawing tube and the exact length of the dendritic segment calculated by placing a thread along the drawing and then measuring the thread length. Spine density was expressed as the number of spines per 10 μm . No attempt was made to correct for spines hidden beneath or above the

dendritic segment so the spine density values are likely to underestimate the actual density of the dendritic spines.

2.3.4. Statistical Analyses

Owing to the absence of senescent female groups, analyses of variance (ANOVAs) were done separately on male and female groups. For the anatomical analyses two-way ANOVAs were performed with age and experience as factors. Follow-up tests were conducted using Fisher's LSD.

2.4. RESULTS

2.4.1. Behavioral Observations

The juvenile and young adult rats were frequently observed interacting with the objects and moving about the cage. In fact, they became extremely agile and if offered food treats (such as Froot Loops™) anywhere along the hardware cloth walls they could rapidly locomote up and down the runways and walls. In contrast, many of the senescent rats were rarely observed to leave the floor of cage and if they did so, they would usually climb only to the first shelf. After a lifetime of cage living the animals clearly were motorically disadvantaged and quite timid about climbing very high. They did interact with the objects, however, and when objects were changed in their environment they actively investigated the new ones. We should note that a minority of the aged rats did investigate the entire cage and were often observed on upper levels.

One unexpected observation was that given a choice, the rats in the juvenile and young adult groups preferred to sleep in cages that were hung a meter or more off the floor. They were rarely observed sleeping on the cage floor. If nesting material such as paper towel was available on the condo floor both male and female animals would carry it up to the hanging cages where they built nests. The aged animals generally slept in groups at the back of the condominium at floor level.

Finally, we routinely watched the animals in both the light and dark periods with a goal of seeing obvious sex differences in behavior in the condominiums but, like Juraska and Meyer (1986) before us, we saw little difference except that the young males engaged in more rough and tumble play than the females.

2. 4. 2. Anatomical results: Male Rats

2. 4. 2. 1. Gross morphology: brain and body weight

One consistent result of our studies is that enriched housing produces reliable increases in brain weight, which appear to be independent of body weight (Table 2.1). Thus, in the current study, there was an increase in brain weight in enriched rats, regardless of age. The overall increase was about 5%, although it ranged from 7% in the juvenile animals to 3% in the young adult animals. A two-way analysis of variance (Experience X Age) revealed a main effect of experience ($F(1,38)=13.7, p=.0007$) but there was no main effect of age ($F(2,38)=2.2, p=.12$) nor was there an interaction ($F(2,38)=0.6, p=.55$).

Table 2. 1. Summary of brain weights for the male rats

Group	Experience	
	Cage-Housed	Condo-Housed
Juvenile	2.17±.02 (n=5)	2.32±.04* (n=5)
Young Adult	2.17±.03 (n=7)	2.24±.03 (n=5)
Aged	2.23±.04 (n=9)	2.32±.03* (n=13)

Numbers refer to mean weight in grams ± the standard errors
*Differs significantly from the same age cage- housed group
(p<.05)

The increase in brain weight in enriched rats was not related to body weight in any simple manner (Table 2.2). There was no effect of enrichment on body weight in the juvenile or aged rats although enrichment decreased body weight in the young adults by nearly 200 gm. It may be important to note that the effect of enrichment on brain weight was the smallest in the young adult group, which may be related to the rather large drop in body weight in the enriched rats at this age. (Body weight was matched in the groups when the enrichment began.) In contrast to the brain weights, there

was also an age-related effect on body weight. This reflects the common observation that male rats continue to gain weight throughout their lifetime. Thus, because the animals were different ages at the end of the experiment, it is not surprising that there are weight differences.

Table 2. 2. Summary of body weights for the male rats

Group	Experience	
	Cage-Housed	Condo-Housed
Juvenile	496±19	496±27
Young Adult	652±23	473±4*
Aged	619±38	683±15

Numbers refer to means ± standard errors.

*Differs significantly from same age cage-housed group ($p < .05$).

A two-factor (Experience X Age) analysis of variance showed main effects of experience ($F(1,29)=4.47, p=.04$) and age ($F(2,29)=24.0, p<.0001$) as well as the interaction ($F(2,29)=16.9, p<.0001$). (The body weights of nine aged rats were not included in the analysis because these weight records were misplaced.) Posthoc tests (Fisher's LSD) showed a significant difference in the young adult groups ($p < .05$).

2. 4. 2. 2. Dendritic arborization

There was a dramatic increase in dendritic arborization in condo-housed rats at all ages and in both parietal and occipital cortex (Figures 2.2 and 2.3). This increase was remarkable, ranging from 15-25% more branches in the condominium-housed groups, depending on the measure (apical versus basilar field) and location (parietal versus occipital). There was also an age-related effect as the aged rats had less arborization than the younger groups. Tables 2.3 and 2.4 summarize the mean (\pm SEM) for the branching and length measures in the parietal and occipital areas respectively.

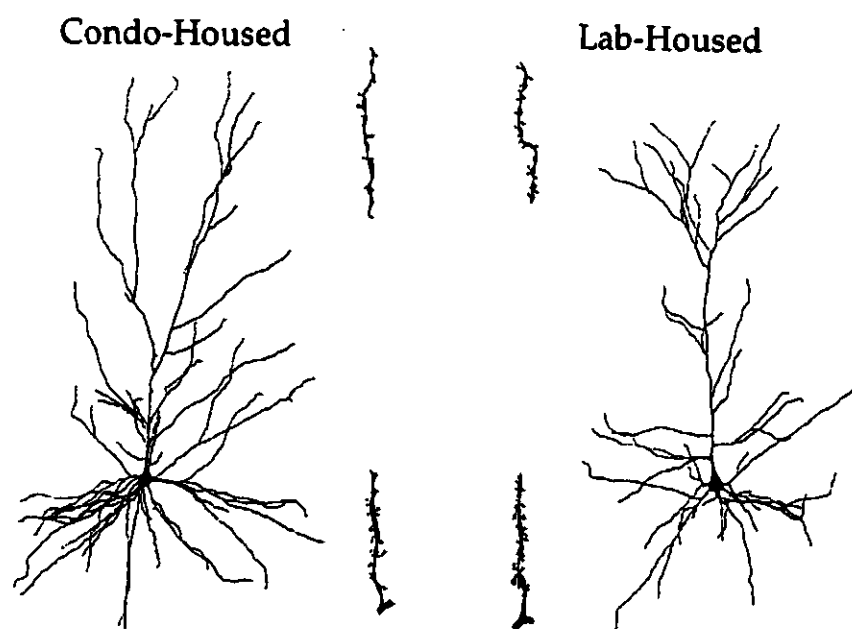


Figure 2. 2. Drawings of representative layer III pyramidal neurons from area Par1 (Zilles, 1985) in young adult rats that were housed in the

condominiums from weaning until about four months of age. Cells from the condominium-housed animals showed more dendritic arbor but a reduced spine density relative to cells from lab-housed animals.

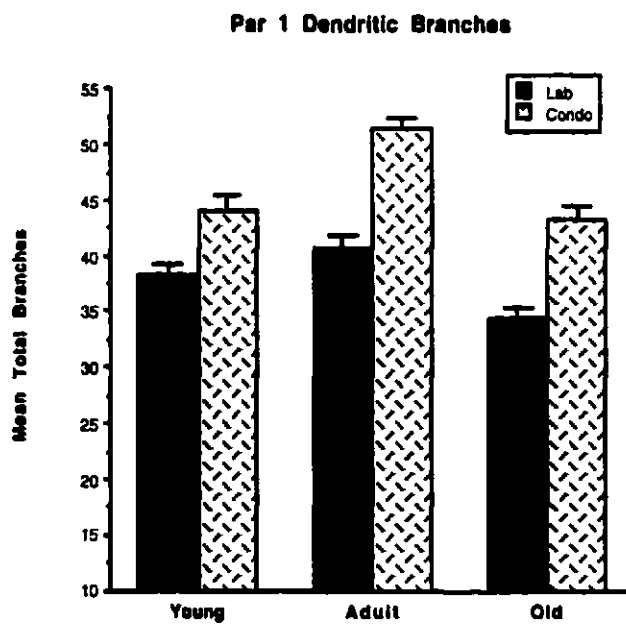
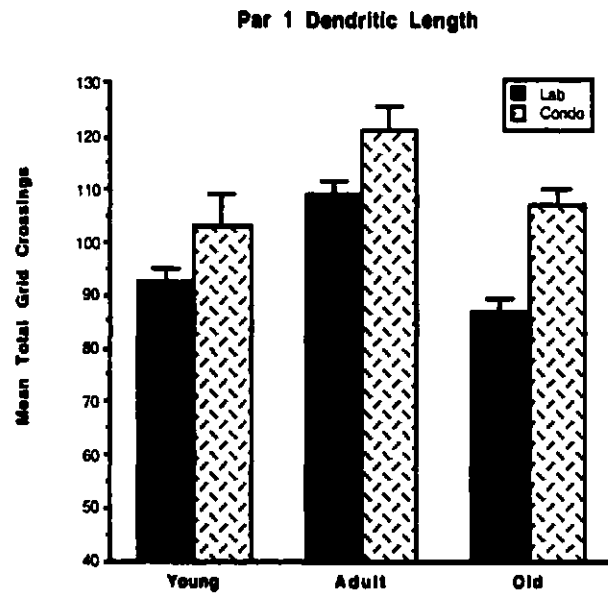


Figure 2.3. Summary of the differences in dendritic length (top) and dendritic branching (bottom) of the basilar field of layer III pyramidal cells in parietal cortex of male rats. Rats housed in the condominiums (Condo) had greater dendritic arbor regardless of age of the experience.

Table 2. 3. Summary of dendritic length in parietal cortex for the male rats

Group	Apical		Basilar	
	Cage	Condo	Cage	Condo
Juvenile	76.4±5.3	95.0±4.3*	92.3±2.5	102.9±6.2*
Adult	71.6±1.6	91.4±3.5*	109.0±2.4	121.0±4.8*
Aged	71.4±2.2	82.7±1.4*	86.9±2.6	107.5±3.1*

Numbers refer to mean grid crossings where grids are 20 μm apart.

*Differs significantly from same age cage-housed group ($p < .05$ or better).

Table 2. 4. Summary of dendritic length in occipital cortex for the male rats

Group	Apical		Basilar	
	Cage	Condo	Cage	Condo
Juvenile	66.3±2.7	76.3±2.1*	93.9±2.4	100.0±3.7*
Adult	58.1±2.2	73.2±2.6*	75.9±4.9	102.4±4.3*
Aged	66.4±3.3	76.7±1.7*	99.2±2.4	107.3±2.0*

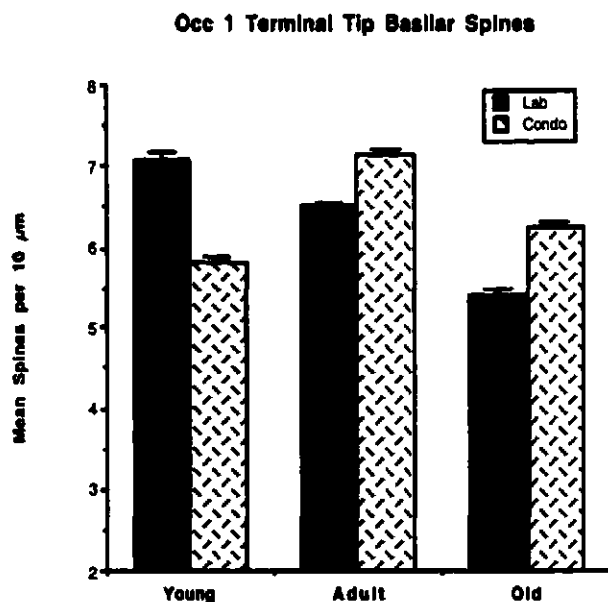
Numbers refer to mean grid crossings where grids are 20 μm apart.

*Differs significantly from same age cage-housed group ($p < .05$ or better).

Separate two-way analyses of variance (Age and Experience as factors) were performed on each of the apical and basilar fields in each of the parietal and occipital cortex and on each of the branching and length measures. In all cases there were significant main effects of enrichment (p 's $< .001$ or better). There were also significant effects of age for apical and basilar fields of the parietal cortex and the basilar field in the occipital cortex (p 's $< .01$ or better). The only significant interactions were for the branching and length measures of the basilar dendrites in occipital cortex (p 's $< .01$). These interactions reflected the relatively larger effect of experience on the adult than the young or old groups.

2.4.2.3. Spine density

In contrast to the general increase in dendritic branching and length, there was an interaction between age and qualitative change in spine density. Thus, whereas enriched young adult and aged rats showed *increased* spine density, enriched juvenile rats showed *decreased* spine density (Figure 2.4; Tables 2.5 & 2.6). This result was seen in the terminal branches of both the apical and basilar branches in both parietal and occipital cortex.



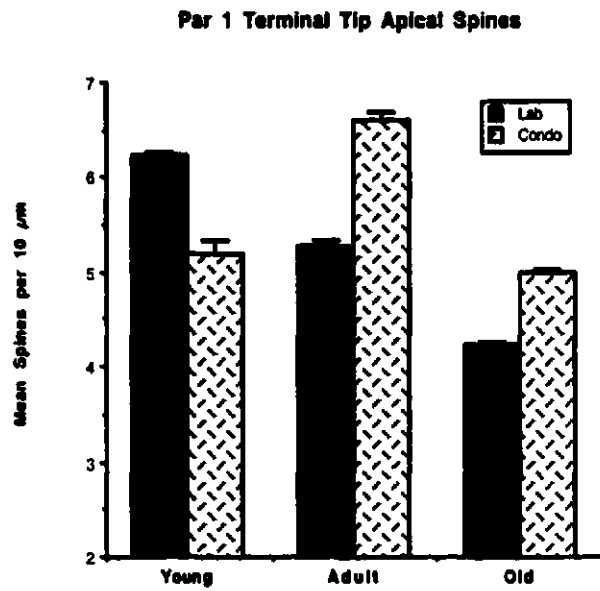


Figure 2.4. Summary of the differences in spine density of the basilar (top) and apical (bottom) terminal tips from layer III pyramidal cells in occipital and parietal cortex (respectively) of male rats. Condominium housing led to decreased spine density in young animals and increased spine density in adult and aged animals

Table 2. 5. Summary of spine density in parietal cortex for the male rats

Group	Apical Terminal Tip		Basilar Terminal Tip	
	Cage	Condo	Cage	Condo
Juvenile	6.24±.04	5.18±.17*	6.69±.08	5.85±.04*
Adult	5.29±.15	6.61±.07*	6.67±.04	7.15±.05*
Aged	4.24±.04	5.01±.03*	5.23±.09	6.05±.02*

Numbers refer to mean spines per 10 μm \pm standard errors.

*Differs significantly from same age cage-housed group ($p < .01$).

Table 2. 6. Summary of spine density in occipital cortex for the male rats

Group	Apical Terminal Tip		Basilar Terminal Tip	
	Cage	Condo	Cage	Condo
Juvenile	6.05±.08	5.48±.23*	7.09±.08	5.82±.05*
Adult	6.72±.17	6.56±.05	6.51±.04	7.16±.07*
Aged	4.46±.06	5.29±.05*	5.42±.05	6.25±.07*

Numbers refer to mean spines per 10 μm \pm standard errors.

*Differs significantly from same age cage-housed group ($p < .01$).

In addition, just as we observed for the results of dendritic branching, there was an age-related change in spine density in the cage-housed rats. Thus, the aged cage-reared rats had a lower spine density than the other two cage-reared groups (Tables 2.5 & 2.6). Taken together, the lower dendritic arborization and lower spine density in the aged rats implies that the aged animals suffered significant synaptic loss over their lifetime.

Two-way analyses of variance with age and experience as factors were performed on each of the apical and basilar terminal tips in each of parietal and occipital cortex. There was a main effect of age on all measures ($p's < .01$). There was a significant main effect of experience only for the apical spine density in parietal cortex ($p < .01$), but there were significant interactions between age and experience on all measures ($p's < .01$ or better), which reflects the qualitative difference in spine density changes in the juvenile and older rats. This was due to a lower spine density in the aged rats than in the other two groups.

2. 4. 3. Anatomical Results: Female Rats

Although there were no aged female subjects, the overall results for the female rats were essentially identical to the male rats. There was, however, one technical difficulty with the analysis of the occipital cells in the female brains. The occipital cells from the adult lab-housed females were inadvertently not drawn at the time that all other sections were drawn. This was discovered nine years later, at which time there had been some fading of

the staining of the terminal tips. We judged it unwise to try to draw these cells to compare that group of animals to the other three groups of females or to the males. Given that there were no differences between the juvenile and young adult female lab-housed animals in parietal cortex, we felt confident that we could perform simple ANOVAs comparing the juvenile cage-reared, juvenile condo and adult condo groups for the occipital analyses.

2.4.3.1. Gross morphology: brain and body weight

As in male rats, the female rats housed in the condominiums had increased brain size. Perhaps surprisingly, it was a somewhat larger effect in the females than in the males, averaging about 9% (Table 2.7). One advantage in the analysis of the females, however, is that condominium housing had absolutely no effect on body weight (Table 2.8). Thus, there is no possible confound between changes in brain and body weights.

Table 2.7. Summary of brain weights for the female rats

Group	Experience	
	Cage-Housed	Condo-Housed
Juvenile	1.98±.03 (n=5)	2.13±.02* (n=5)
Young Adult	1.92±.02 (n=7)	2.11±.03* (n=5)

Numbers refer to means ± standard errors.

*Differs significantly from same age cage-housed group (p<.05).

Table 2. 8. Summary of body weights for the female rats

Group	Experience	
	Cage-Housed	Condo-Housed
Juvenile	295±19 (n=5)	298±25 (n=5)
Young Adult	359±18 (n=7)	25±17 (n=5)

Numbers refer to means ± standard errors.

Analysis of variance on the brain weight showed a significant main effect of experience ($F(1,18)=36.2$, $p<.0001$) but not of either age ($F(1,18)=2.4$, $p=.14$) or the interaction ($F(1,18)=0.73$, $p=.40$). Analysis of variance on body weight revealed a main effect of age ($F(1,18)=4.9$, $p=.04$), but not of either experience ($F(1,18)=0.5$, $p=.47$) or the interaction ($F(1,18)=0.8$, $p=.38$).

2. 4. 3. 2. Dendritic branching

As in male rats there was a general increase in dendritic arborization for the condominium-housed female rats in both the parietal and occipital cortex, but in contrast to the males, there was no increase in the dendritic arbor in the basilar dendrites of the juvenile -enriched rats (Figure 2.5; Tables 2.9 & 2.10).

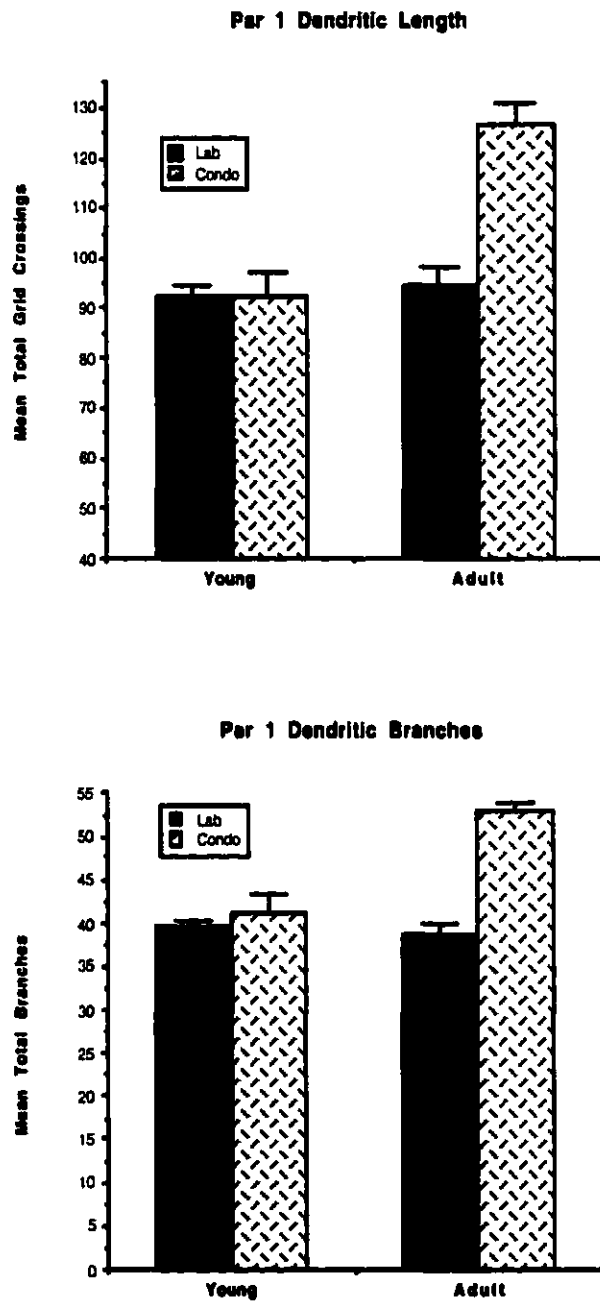


Figure 2.5. Summary of the effect of condominium housing on the dendritic length (top) and branching (bottom) of layer III pyramidal cells in Par 1 of female rats. There was no effect of experience in the young animals but a large increase in dendritic branching in the adult animals.

Table 2.9. Summary of dendritic length in parietal cortex for the female rats

Group	Apical		Basilar	
	Cage	Condo	Cage	Condo
Juvenile	75.2±2.0	89.4±2.5	92.3±2.4	92.4±4.8
Young Adult	80.4±4.5	101.4±2.8*	94.8±3.6	126.7±4.6*

Numbers refer to mean grid crossings where grids are 20 μm apart.

*Differs significantly from same age cage-housed group (p<.05 or better).

Table 2.10. Summary of dendritic length in occipital cortex for the female rats

Group	Apical		Basilar	
	Cage	Condo	Cage	Condo
Juvenile	75.6±3.3	73.6±1.8	88.2±3.9	87.7±5.5
Adult	NA	79.5±4.4	NA	112.5±3.4*

Numbers refer to mean grid crossings where grids are 20 μm apart.

*Differs significantly from cage-housed group (p<.01).

Analyses of variance were performed with age and experience as factors

for each measure of the apical and basilar fields of parietal cortex and, as noted above, simple analyses of variance were performed for the occipital cells. For the parietal cortex there were significant main effects of experience on all measures but significant main effects of age, and the interactions, only on the basilar measures ($p's < .01$ or better). For the occipital cortex, there was a significant main effect for both the basilar measures, with the adult enriched group having longer dendrites than the other groups, which did not differ. The apical data were less consistent, however. There was a significant main effect for branching, with the adult enriched group being branchier than the other groups, but there was no effect on the dendritic length.

2.4.3.3. Spine density

There was a qualitative difference in the effect of experience on the spine density in male and female rats. In the parietal cortex there was a *decrease* in spine density in the condo groups at both ages (Table 2.11). In the occipital cortex, there were no effects on spine density in the apical terminal tip but for the basilar tip the results were similar to those observed in the males: adult condominium-housed females showed an *increase* in spine density whereas juvenile condominium-housed females showed a *decrease* in spine density (Table 2.12).

Table 2.11. Summary of spine density in parietal cortex for the female rats

Group	Apical Terminal Tip		Basilar Terminal Tip	
	Cage	Condo	Cage	Condo
Juvenile	5.4±.16	4.8±.27	6.1±.05	5.2±.05*
Adult	5.8±.04	5.4±.09	6.8±.05	6.4±.07*

Numbers refer to mean spines per 10 μm \pm standard errors.

*Differs significantly from same age cage-housed group ($p < .05$).

Table 2.12. Summary of spine density in occipital cortex for the female rats

Group	Apical Terminal Tip		Basilar Terminal Tip		
	Cage	Condo	Cage	Condo	
Juvenile		5.9±.12	5.9±.28	6.1±.08	5.4±.07*
Adult		NA	5.8±.08	NA	6.7±.06*

Numbers refer to mean spines per 10 μm \pm standard errors.

*Differs significantly from cage-housed group ($p < .01$)

NA, data not available.

Two-way analyses of variance with age and experience as factors were performed on each of the apical and basilar terminal tips for the parietal cortex. There were significant main effects of age and experience on both parts of the cells but only the basilar interaction was significant ($p's < .01$). The simple ANOVA on the occipital cortex revealed a significant main effect on the basilar but not the apical tips.

2. 5. DISCUSSION

There were four principal findings of these studies. First, experience produces multiple changes in brain morphology. Second, there is a qualitative difference in the effect of experience in spine density at different ages. Third, although both male and female brains showed large experience-dependent changes in brain morphology, there are qualitative sex differences. Fourth, aged rats showed large changes in dendritic morphology that were related both to aging and experience. We shall consider each of these results in turn.

2. 5. 1. Experience and the changing brain

It is already well established that experience produces profound changes in the brain, ranging from changes in neural and glial structure to neurochemical and blood vessel changes (for reviews, see Greenough, Black & Wallace, 1987; Greenough & Chang, 1988; Juraska, 1990; Kolb & Whishaw, 1998; Sirevaag & Greenough, 1988). In the current study we found increased

brain weight, increased dendritic arborization, increased dendritic length, and altered spine density. With the exception of spine density, all of the measures showed qualitatively similar changes across the life span.

2. 5. 2. Qualitative differences in spine density

Although it is generally believed that enriched housing leads to increased spine density there are actually very few studies showing this effect (Globus et al., 1973; Schapiro & Vukovich, 1970). Curiously, both of these studies used young animals; the Globus study used only males and sex was not specified in the Schapiro study. The Globus study used weanling animals as we did and the enriched housing lasted 30 days. They ran four experiments and failed to find any spine density changes in two of the experiments. Schapiro and Vukovich used newborn animals that were handled and stroked, shaken on a mechanical shaker, placed in warm and cold water and on warm and cold metal and finally subjected to electric shock, noise and flashing lights. When the animals were killed at eight days of age, there was an increase in spine density. This procedure is very different from the current one, and presumably involved considerable stress, which may have influenced the results. In fact, we have tactilely stimulated newborn rats daily from birth until weaning and found them to show a decrease in spine density and, like Schapiro and Vukovich, no change in dendritic length (Kolb, Gibb, Gorny & Ouellette, 1996).

Our finding of a decrease in spine density in young animals is not

without precedent. Several studies have shown in chicks that neurons in the hyperstriatum show a chronic decrease in spine density when animals are imprinted to visual or auditory stimuli (Bock & Braun, 1998; Wallhauser & Scheich, 1987). In contrast to this result, Patel, Rose and Stewart (1988) trained chicks on the passive avoidance task and found an increase in spine density 25 h after training. Thus, taken together the chick experiments show that there is an increase in spine density 25 hours after training but there is a decrease seven days after training. The simplest conclusion from the chick studies is that the novel stimulation may cause an initial rapid increase in spine density, followed by a pruning. If we extrapolate to the current study we might predict that the juvenile animals showed an increase in spine density over the first hours or days in the condominiums, followed by a synaptic pruning. This could account for the difference between the Globus and current studies. The critical experiment would be to examine brains of juvenile animals housed in the condominiums for varying periods of time.

2. 5. 3. Sex and the changing brain

There is considerable evidence that there are sex differences in the structure of the adult male and female rat cortex (e.g., Juraska, 1990; Kolb & Stewart, 1991; 1995; Pfaff, 1966; Stewart & Kolb, 1988; 1994; Yanai, 1979). In particular, the cortex of the male tends to be thicker than that of the female. Furthermore, there are areal-dependent differences in dendritic arborization. For example, cells in the medial frontal region have larger dendritic fields in

males whereas the fields are larger in cells in the orbital frontal region in females (Kolb & Stewart, 1991). One explanation for some of the sex differences in cortical neuronal architecture may be that males and females respond differently to experience. Indeed, in an important and novel set of experiments Juraska and her colleagues have demonstrated sex differences in the effects of enriched housing on the cortex of rats (e.g., Juraska, 1984; 1986; 1990). In her studies Juraska showed that when male and female rats were placed in complex environments from weaning until 55 days of age, males showed a much larger change in both pyramidal and stellate cells in visual cortex than did females. This was a startling result with important implications for understanding sex differences in the behavior of both rats and humans. In addition, Juraska found that whereas neocortical areas appeared to be more sensitive to experience in male rats, similar experience produced larger changes in the dentate gyrus of the hippocampal formation in female rats (Juraska et al., 1985; 1989).

The results of the current study are consistent with the conclusions of the Juraska study but, in addition, we show that the sex differences are also found in spine density and that they are age-dependent. In young animals, we found that although males and females both showed a drop in spine density with complex housing, the effect on dendrites was sexually dimorphic: males showed a much larger increase than females and females actually showed no effect in the basilar fields in either parietal or occipital cortex. In adult animals we found that both sexes showed increases in

dendritic arborization but this time the effect of experience on the spines was dimorphic: Males showed an increase in spine density but females did not. The tendency for males to show plastic increases in spine density that are not seen in females is consistent with our findings that male, but not female, rats with frontal lesions around seven days of age show compensatory increases in spine density (Kolb & Stewart, 1995). Thus, there may be a fundamental difference in the way in which cortical neurons in males and females respond to different experiences.

2. 5. 4. Aging and the changing brain

One observation of the current study was that although the dendritic arborization of the neurons of aged rats was comparable to that of the younger animals, there was a dramatic decrease in spine density in the aged cage-housed rats. Nonetheless, these neurons appear to be very responsive to experience. The proportional increase in spine density observed in the condominium-housed aged rats relative to the cage-housed rats was larger in the aged rats than in the young adults, especially in occipital cortex. It is likely that the reduced spine density, and presumably synaptic number, reflects a lifetime of relative deprivation in the aged animals. It is interesting, therefore, that the effects of a lifetime (over two years) of restricted perceptual and motoric experience can be reversed by enriched housing for as little as three months. We have found in parallel studies that if animals are trained on various cognitive or motor tasks throughout their lifetime this apparent

synaptic pruning is prevented and, in fact, aged animals may actually show an increase in both dendritic branching and spine density relative to young adults with similar experience (e.g., Miklyeva, Whishaw & Kolb, 1997). It has been speculated that this relative increase in aged animals may reflect a mechanism for accommodating for neuron death (e.g., Buell & Coleman, 1985). It may be that such compensation occurs only if animals have a need to create synapses, such as when they have novel experiences. Although speculative, an investigation of this possibility may help clear up apparent discrepancies in the literature regarding morphological changes in aged animals (e.g., Flood, 1993).

3. EXPERIMENT TWO: EXPERIENCE AND THE INJURED BRAIN

3. 1. ABSTRACT

The current study is a compilation of 3 experiments which detail the effect of complex housing on rats with frontal cortex removals at either postnatal day 2 (P2) or postnatal day 7 (P7). Experiment 2A and 2B examine recovery from P2 injury (an age when spontaneous functional recovery is limited) when the animals are placed in complex housing at weaning or in adulthood, respectively. In Experiment 2C, animals with P7 frontal removals (an age when spontaneous functional recovery is high) were placed in complex environments at weaning to determine if further functional improvement was possible. Behavioral assessment on the Morris Water task was performed in all of the experiments and additional assessment on the Whishaw reaching task was included in Experiment 2C. Complex housing can improve behavioral recovery after early cortical lesion but its effectiveness is age-dependent. The earlier environmental intervention begins after injury, the more effective it is in improving functional recovery.

3. 2. INTRODUCTION

One of the key principles of behavioral neuroscience is that experience can modify cortical structure, even long after brain development is complete. Indeed, it is generally assumed that structural changes in the brain accompany various forms of enduring behavioral change including memory storage. Such structural changes include increases in brain size, cortical thickness, neuron size, dendritic branching, spine density, synapses per neuron, and glial numbers (e.g., Bailey & Kandel, 1993; Greenough & Chang, 1988; Kolb & Whishaw, 1998). Most laboratory studies of experience-dependent change manipulate experience either by providing special training or by housing animals in specific types of environments. For example, laboratory animals can be trained to make specific complex movements, such as reaching through a slot for food, or they can be placed in complex environments. In the former case there are changes in the morphology of cells in specific regions, such as primary motor cortex, whereas in the latter case the cellular changes are more global, presumably reflecting the more global activation of cerebral structures.

There have been many studies of the effects of various types of experience on functional outcome after cerebral injury in laboratory animals but the results have been inconsistent and generally disappointing (for reviews see Shulkin, 1989; Will & Kelche, 1992). In the mid 1980s Kolb and his colleagues began a series of experiments designed to look at the effect of

enriched experience on recovery from brain damage sustained at different ages (e.g., Kolb & Elliott, 1987; Kolb & Gibb, 1991). In the course of these experiments it became obvious that experience had effects on the uninjured brain that varied both with age at the time of experience as well as the details of the experience and the sex of the animal. This led us to examine in detail the effects of experience on the developing, normal, brain (e.g., Kolb, Gibb & Gorny, 2001). Armed with this knowledge, the current set of studies returns to the original question regarding the effects of experience on recovery from frontal cortical injury in infancy.

The current paper describes the results of three experiments (2A, 2B, & 2C). The first two examine the effect of experience on recovery from frontal lesions at two days of age. Previous studies had shown that rats with frontal injuries at this time have a poor behavioral outcome (e.g., Kolb, 1987), so we anticipated that environmental stimulation would potentiate functional recovery. The key difference between the experiments is that in Experiment 2A the animals were placed in complex environments for three months beginning at weaning whereas in Experiment 2B the animals were placed in a complex environments for three months beginning in adulthood. We anticipated that animals with stimulation during development might benefit more from the experience than animals with similar experience in adulthood. The third experiment looked at the effect of experience on recovery from frontal lesions at seven days of age. We had shown previously that rats with lesions at this age showed considerable recovery of function

(e.g., Kolb & Whishaw, 1981) so we asked first whether the brains of these animals were capable of further plasticity. In addition, because we had found that there was a sex difference in the behavioral and anatomical sequelae of frontal cortex lesions at seven days of age, we asked whether any experience-dependent changes might be sexually dimorphic. Like Experiment 2A, the animals were placed in complex environments for three months, beginning at weaning. Littermate control animals were group-housed in standard hanging laboratory cages during the same periods in all of the experiments.

3. 3. MATERIALS AND METHODS

3. 3. 1. Experiment 2A

3. 3. 1. 1. Subjects

The study was done with 37 Long-Evans rats (four litters) derived from Charles-River strains, which were divided into four groups: 1) lab control (4 Male (M), 3 Female (F)), lab frontal (4M, 6F), enriched control (5M, 3F), and enriched frontal (6M, 6F). The rats in each age group were assigned to either the lab or enriched housing such that body weight was approximately equal in the lab and enriched groups and that approximately equal numbers of animals in each of the lesion and treatment groups came from the same litter.

3. 3. 1. 2. Surgical procedures

The animals were anesthetized on the day after birth (postnatal day two, P2) by cooling them in a Thermanon cooling chamber until their rectal

body temperatures were in the range of 18-20°C. For the frontal rats the frontal bone was removed by cutting it with iris scissors, and frontal decortication was achieved by gentle aspiration. The intent was to remove the medial subfields of the prefrontal cortex including the presumptive Zilles' (1985) regions Cg1, Cg3, and PL as well as the medial portion of Fr2 of the motor cortex. As soon as medial frontal decortication was achieved, the animals' scalps were sutured with silk thread. The normal control group animals were anesthetized in the same manner, and the skin was incised and sutured. The control animals were identified by removing an outer toe on the right back foot.

3. 3. 1. 3. Enrichment procedures

The rats were reared with their mothers in 22 X 44 X 18 cm Plexiglas cages with corn cob chip bedding until they were 22 days of age. The lab-reared animals were housed in 65 X 26 X 18 cm stainless steel hanging cages (3-4 same sex per cage) in a busy animal facility containing about 500 other rats. The enriched animals were weaned and moved to large pens measuring 1.5 X 1.5 X 0.5 m. The pen floor was covered with 10 cm of sawdust bedding. The pen was filled with a half a bale of straw, lengths of PVC pipe, branches, discarded children's toys, and other laboratory 'junk'. The objects were changed when the cages underwent their weekly cleaning. There were two similar pens with 4-6 same-sex animals housed in each. After three months the animals were placed in standard laboratory cages for an adaptation period

of two days before behavioral testing began. The brains were harvested two weeks later. Throughout the experiment the animals were on a 12:12 h light/dark schedule and maintained on ad lib access to rat chow and water.

3.3.1.4. Behavioral methods

The method used in the Morris Water task was similar to that described elsewhere (Sutherland et al., 1983) and is based on the original task described by Morris (1981). The maze was a circular pool (1.5 m diameter x 0.5 m deep) with smooth white walls. The pool was filled with approximately 20°C water, and mixed with 1 L of skim milk powder or just enough to render the water opaque. A clear plexiglas platform (11 x 12 cm) was placed in a constant position inside the pool approximately 12 cm from the wall. The water level was adjusted so that the platform stood 2 cm below the surface of the water. The platform was invisible to a viewer outside the pool and to a rat swimming in the water. A trial consisted of placing a rat into the water at one of four locations (north, south, east, or west) around the pool's perimeter. Within a block of four trials each rat started at the four locations in a random sequence, and each rat was tested for four trials a day over five consecutive days, for a total of 10 trial blocks. If on a particular trial a rat found the platform, it was permitted to remain on it for 10 seconds. A trial was terminated if the rat failed to find the platform after 90 seconds. Each rat was returned to its holding cage for approximately five minutes before the next trial commenced. The swim path for each rat on every trial was traced by the

experimenter and latency to find the platform was recorded. The heading error was calculated manually from the drawings.

3. 3. 1. 5. Anatomical methods

Following the conclusion of the behavioral testing the animals were given an overdose of sodium pentobarbital and intracardially perfused with 0.9% saline. The brains were removed, weighed, and immersed whole in 20 ml of Golgi-Cox solution. The brains were left in the solution for 14 days before being placed in a 30% sucrose solution for two to five days, cut frozen on a cryostat at 120 μm , and developed using a procedure described by Kolb and McLimans (1986). Unfortunately, owing to unexpected technical difficulties arising from high humidity at the time of staining, the staining was uneven and it was not possible to draw dendritic fields in many of the brains. Thus, because the group sizes became too small to do a useful analysis, we measured cortical thickness as an estimate of the effects of lesion and experience.

3. 3. 2. Experiment 2B

3. 3. 2. 1. Subjects

The study was done with 44 Long-Evans rats derived from five litters of Charles-River strains, which were divided into four groups: 1) lab control (6M, 5F), lab frontal (7M, 6F), enriched control (5M, 5F), and enriched frontal

(6M, 4F). Rats were assigned to experimental conditions as in Experiment 2A.

Surgical procedures were as in Experiment 2A.

3.3.2.2. Enrichment procedures

The rats were reared with their mothers as in Experiment 2A but after weaning they all were housed in 65 X 26 X 18 cm stainless steel hanging cages (3-4 per cage) for three months before being placed into the complex housing conditions. The complex housing took place in large vertically organized pens measuring 63 X 148 X 187 cm. Three of the walls (sides and front) were made of hardware cloth. The back wall was made of plywood covered with blue arborite, as was the ceiling and floor. Two stainless steel cages (22 X 26 X 18 cm) were attached to the upper part of the front wall and another was placed on its side on the cage floor. There also were runways attached to the back wall, which allowed animals to navigate from the floor to a shelf near the top without having to run up the hardware cloth walls. There were two similar pens with 4-6 same-sex animals housed in each. The pen floor was covered with about 10 cm of sawdust bedding. The pen was filled with similar objects to Experiment 2A. The objects were changed weekly. The animals were left undisturbed except for daily feeding and weekly cleaning of the pen.

3. 3. 2. 3. Behavioral methods

Animals were trained in the Morris task as in Experiment 2A but with three differences. First, because the task used in Experiment 2A proved quite difficult, the maze was moved to a smaller room that had additional extramaze cues. We had found in other studies that this made the task easier both for control and lesion animals. Second, rather than calculate the heading errors from the manual tracings of swim path, errors were measured. Errors were determined by counting deviations off a direct path from each starting point to the platform. Specifically, the errors were calculated on the traced swim path using a 1.5 cm wide strip of paper arranged so that it covered the platform and the starting point. Any swim path that was lying outside the direct path was counted as one error. Third, the rats were given nine trial blocks with the platform in one location before it was moved to the diagonally opposite quadrant for the tenth trial block.

3. 3. 2. 4. Anatomical methods

Following the conclusion of the enriched housing the animals were given an overdose of sodium pentobarbital and intracardially perfused with 0.9% saline. The brains were removed and weighed before being immersed whole in 20 ml of Golgi-Cox solution. The brains were left in the solution for 14 days before being placed in a 30% sucrose solution for two to five days, then cut on a Vibratome™ at 200 μm , and developed using a procedure described by Gibb and Kolb (1998).

Layer III pyramidal cells in Zilles' area Par 1 were traced using a camera lucida at 250X. In order to be included in the data analysis, the dendritic trees of pyramidal cells had to fulfill the following criteria: (a) the cell had to be well impregnated and not obscured with blood vessels, astrocytes, or heavy clusters of dendrites from other cells; (b) the apical and basilar arborizations had to appear to be largely intact and visible in the plane of section. The cells were drawn and analyzed using two different procedures. In the first, each branch segment was counted and summarized by branch order using the procedure of Coleman and Riesen (1969). Branch order was determined for the basilar dendrites such that branches originating at the cell body were first order; after one bifurcation, second order; and so on. Branch order was determined for the apical dendrites such that branches originating from the primary apical dendrite were first order and so on. In the second, a Sholl analysis (Sholl, 1956) was performed. For this analysis a transparent overlay of concentric circles spaced 20 μm apart was placed over the neuron drawing by centering the innermost ring in the middle of the cell body. The number of dendrite-ring intersections was counted for each ring and the total number used to estimate total dendritic length in μm (number of intersections X 20). Ten cells were drawn in each hemisphere of each rat. The statistical analyses were done by taking the mean of the measurements on the ten cells for each hemisphere of each subject.

Spine density was measured from one apical dendritic branch in the terminal tuft and one basilar terminal branch. Spine density measures were

made from a segment greater than 10 μm in length, and usually about 50 μm . The dendrite was traced (1000X) using a camera lucida and the exact length of the dendritic segment calculated by placing a thread along the drawing and then measuring the thread length. Spine density was expressed as the number of spines per 10 μm . No attempt was made to correct for spines hidden beneath or above the dendritic segment so the spine density values are likely to underestimate the actual density of the dendritic spines.

3. 3. 3. EXPERIMENT 2C

3. 3. 3. 1. Subjects

The study was done with 40 Long-Evans rats derived from five litters of Charles-River strains, which were divided into four groups of ten rats (5M and 5F per group): 1) lab control, lab frontal, enriched control, and enriched frontal. Rats were assigned to experimental conditions as in Experiment 2A. Surgical procedures were as in Experiment 2A, except that the surgery was performed on P7.

3. 3. 3. 2. Enrichment procedures

The rearing procedures were identical to Experiment 2B except that in order to try to maximize the effect of experience on the brain and behavior, the animals were placed into the complex environments at weaning rather than in adulthood.

3. 3. 3. 3. Behavioral methods

The animals were first trained in the Morris task using the same procedure as Experiment 2B. The animals were then tested in a skilled reaching task. The reaching procedure, developed by Whishaw, Pellis, Gorny, and Pellis (1991), was used to assess the skilled forelimb movements of each rat after being trained to reach for chicken feed pellets in Plexiglas cages (28 cm deep x 20 cm wide x 25 cm high). The front and floor of each cage were constructed with 2 mm bars separated from each other by 1 cm, edge to edge. A tray (5 cm deep x 2 cm wide x 1 cm high) containing chicken feed pellets, was mounted in front of each cage. To obtain food, the rats had to extend their forelimb through the bars, grasp, and retract the food pellet. The food tray was mounted on runners to adjust the distance of the food from the bars. Distance adjustments ensured that each rat could not simply rake the food into the cage. Bars on the floor ensured that if the rat dropped the pellet it would irretrievably lose it and would have to reach again. Rats were trained on the task for a maximum of three weeks before video taping. During the first week, the rats were grouped in pairs in the reaching cages for one hour a day to allow the rats to adapt to their new surroundings. The food deprivation schedule commenced during the first week, and each rat was provided with 15 grams of laboratory rodent food daily following the training period. The rats were subsequently trained individually for one hour each day during the second week whereas during the third week, this training period was shortened to 5–15 minutes a day. Five minutes of continuous

reaching activity for each rat was videotaped and scored when the rats were approximately five months of age. If the rat made a reaching movement (forepaw inserted through the bars, but no food was grasped or it was dropped), it was scored as a "reach". Whereas if the rat obtained a piece of food and consumed it, the movement was scored as a "reach" and a "hit." Scoring was achieved by calculating the percentage of hits to total reaches for each animal's preferred forelimb. Left and right paw reaches and hits were recorded separately.

3. 3. 3. 4. Anatomical methods

The brains were harvested and processed as in the previous experiment. The only difference was that Layer III pyramidal cells were analyzed in both Zilles' areas Par 1 and Occ 1. Dendritic length was analyzed by using the method of Sholl (1956). The number of intersections of dendrites with a series of concentric spheres at 20 μm intervals from the center of the cell body was counted for each cell. Statistical analyses were performed by averaging across all cells per hemisphere. An estimate of mean total dendritic length (in μm) was made by multiplying the mean total number of intersections by 20.

3. 4. BEHAVIORAL RESULTS

3. 4. 1. General Behavioral Observations

As in our previous studies, the enriched animals were frequently observed interacting with the objects and moving about the cages. The pens in Experiment 2A did not allow as much vertical movement (0.5 m versus 1.87 m high) so most exploration in Experiment 2A was on the floor. The animals made tunnels through the straw, carrying the tunneled-out straw to the corners to provide bedding material. The pens in Experiments 2B and 2C allowed the animals to engage in much more vertical activity and the animals took obvious advantage of this opportunity. In fact, they became extremely agile and if offered food treats (such as Froot Loops™) anywhere along the hardware cloth walls they could rapidly locomote up and down the runways and walls. One unexpected observation was that given a choice, the rats in pens preferred to sleep in cages that were hung a meter or more off the floor. They were rarely observed sleeping on the cage floor. If nesting material such as paper towel was available on the pen floor both male and female animals would carry it up to the hanging cages where they built nests. Because both control and frontal rats were housed together, it was not possible to tell if frontal rats carried bedding or made nests but in view of our previous findings that frontal animals do not hoard or nest build, it seems unlikely that they did (e.g., Kolb & Whishaw, 1983).

3. 4. 2. Morris Water Task

3. 4. 2. 1. General observations

Control rats quickly learn that there is a hidden platform in the tank and rapidly learn to swim to the platform from any start location. By the third trial block the control rats in all three experiments asymptoted with an escape latency of about five seconds. As in our previous experiments, we found only a small effect of complex rearing on the water task performance of control animals (e.g., Kolb & Gibb, 1990; Kolb & Elliott, 1987). There were no sex differences in Experiments 2A or 2B, so the data were combined for analyses. Sex was a significant factor in Experiment 2C, however, so the data were not collapsed across sex.

3. 4. 2. 2. Experiment 2A

Rats with P2 frontal lesions are severely impaired at the task and did not learn to swim directly to the platform by the end of the 10 trial blocks. As shown in Figures 3.1 and 3.2, enriched housing improved performance of the P2 frontal-lesion rats. Thus, by the tenth trial block the enriched-frontal rats were locating the platform as quickly as the control animals whereas the cage-reared frontal rats were still taking over 30 sec to find the platform. This difference is further reflected in the heading angle measure. The lab-reared animals were still performing at chance, which is 39°, whereas the enriched-frontal animals had improved to about 20°. This difference could be observed directly too as the lab-reared animals tended to swim a fixed distance from the

pool wall until they bumped into the platform whereas the enriched-frontal animals swam directly towards the platform.

A three-way repeated ANOVA (Lesion X Experience X Trial Block) was performed on the escape latencies. There was a significant main effect of lesion ($F(1,31)=22.2, p<.0001$), treatment ($F(1,31)=5.2, p<.03$), and trial block ($F(9,279)=12.8, p<.0001$). None of the interactions were significant (F 's <1.5 , p 's $>.15$).

For the heading angle analysis a repeated measures ANOVA (Lesion X Experience X Trial Block) was done, using only the heading angles on trial blocks 1 and 10. There was a significant main effect of lesion ($F(1,31)=10.9, p<.002$), and trial block ($F(1,32)=16.1, p<.0004$), but there was no main effect of experience ($F(1,31)=2.5, p=.12$). However, the Experience X Lesion interaction was significant ($F(1,31)=3.8, p<.06$). Post hoc tests on the interaction showed that the two frontal groups differed, as it appears in Figure 2 ($p<.05$).

Experiment 2A: Heading Angles

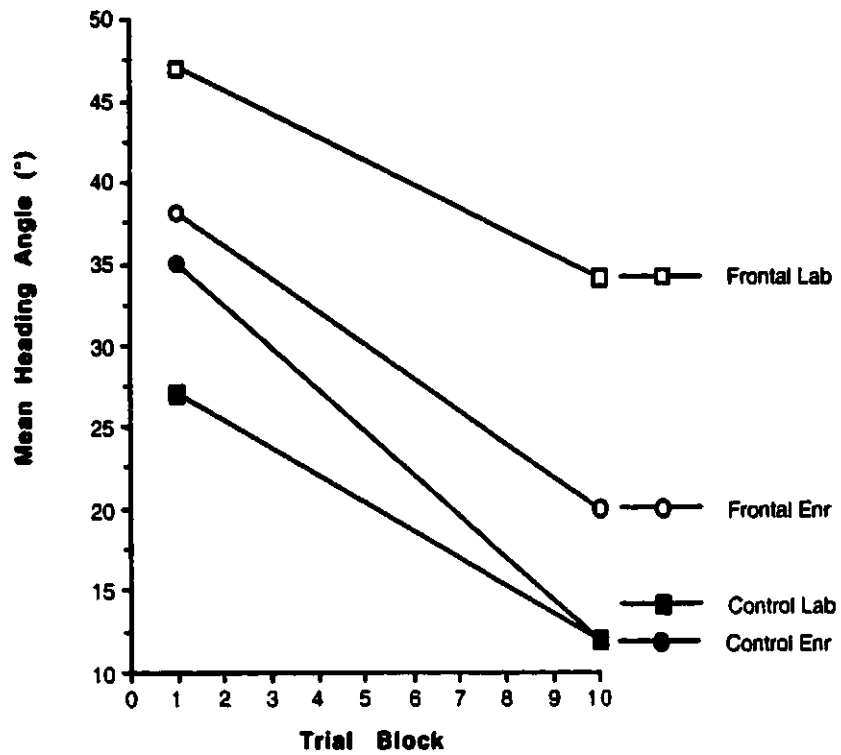


Figure 3.1. The effect of complex housing on water maze performance of control and P2 frontal animals expressed as heading angles. P2 frontal animals showed an improvement in performance after exposure to complex housing.

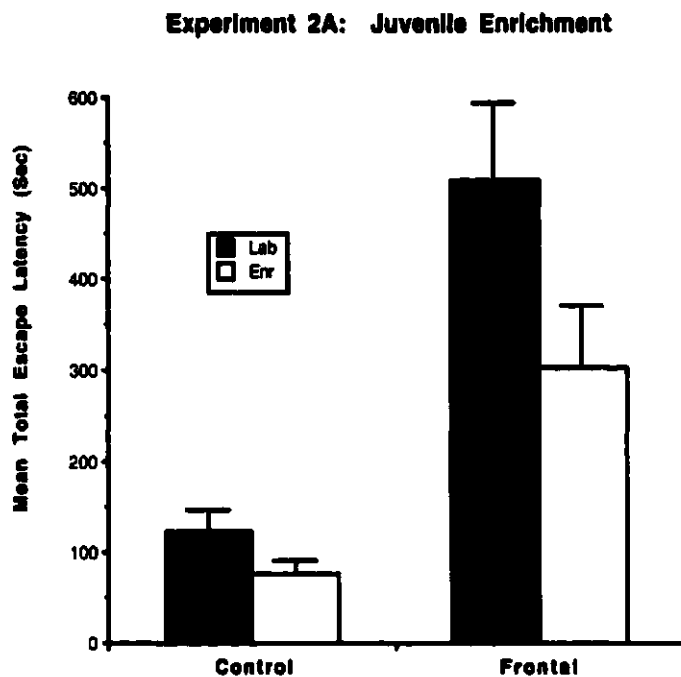


Figure 3.2. Effect of complex rearing on water maze performance expressed as mean total escape latency in seconds. P2 frontals show improved performance after complex housing.

3. 4. 2. 3. Experiment 2B

As in Experiment 2A, the rats with P2 frontal lesions were impaired at the Morris task and even after nine trial blocks the lesion animals were still taking more than twice as long as the control animals to find the platform. Curiously, the enriched P2 frontals initially found the task more difficult than the lab-reared animals. For some unknown reason the complex-reared animals were more disturbed at being placed into the water tank on trial block 1 and, in contrast to the lab-reared animals, most of these animals scratched at

the tank walls for most of the trials. Once the animals began to adopt a searching strategy, however, they quickly learned the task and performed better than the lab reared animals (Figure 3.3 & 3.4).

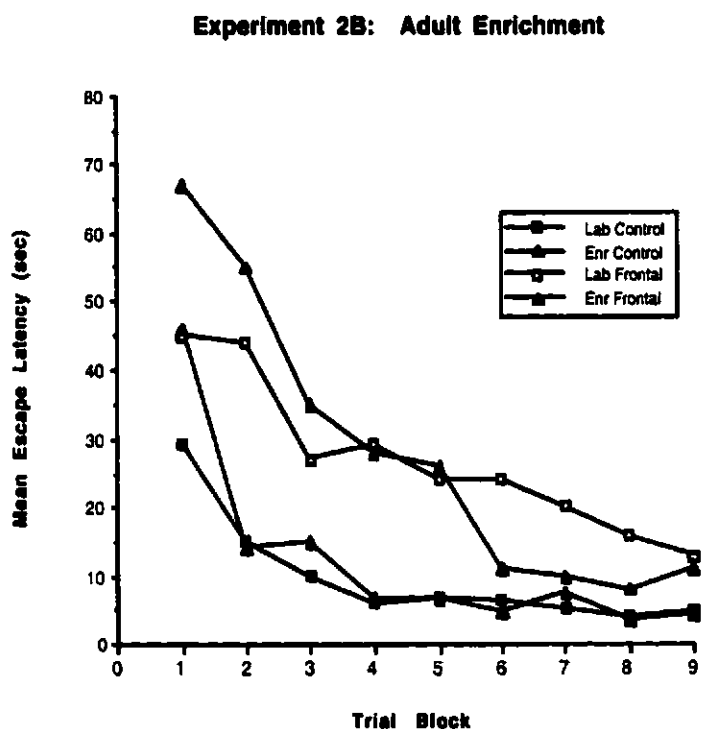


Figure 3.3. Performance of P2 frontals and control animals on the water task. “Enriched” animals exposed to complex housing as adults show marginally improved performance on this task. Performance is measure by mean escape latency in seconds.

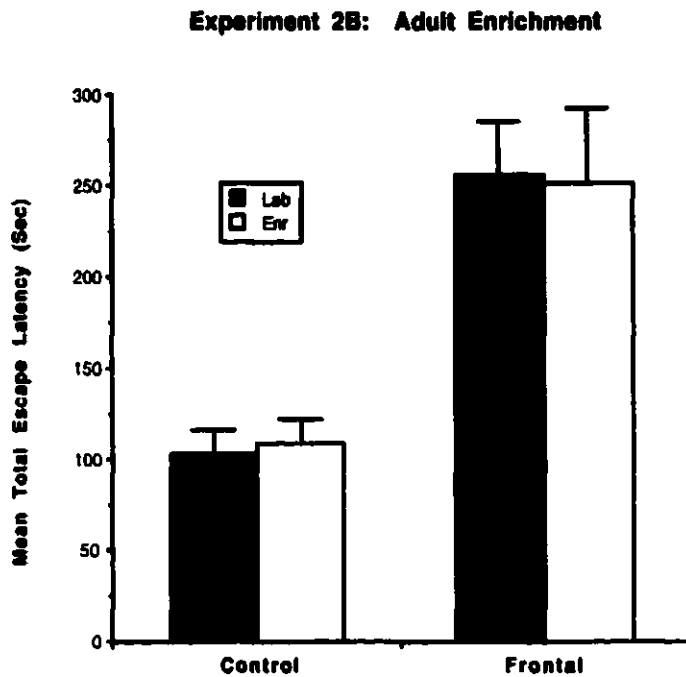


Figure 3.4. Water maze performance of control and P2 frontal lesion animals following exposure to complex rearing in adulthood. No significant differences were detected as a result of complex housing in the performance of the lesion or control groups when the data was summed across all trial blocks.

A three-way repeated measures ANOVA (Lesion X Experience X Trial Block) revealed main effects of lesion ($F(1,39)=33.3, p<.0001$) and trial block ($F(8,304)=53.9, p<.0001$) but not of experience ($F(1,39)=0.3, p=.56$). There was a significant interaction of Lesion X Trial block ($F(8,304)=6.2, p<.0001$) and Experience X Trial Block ($F(8,304)=5.0, p<.0001$). Neither of the other interactions were significant ($F's<1$). The two interactions reflect the fact that

the controls learned the task faster than the lesion animals and that the effect of experience varied by trial block. This latter effect was especially clear in the frontal animals. Comparison of the two lesion groups on trial blocks 5-8 showed that the complex-housed animals were marginally better ($p's < .07$).

In sum, it is clear that the behavioral benefit from complex housing beginning in adulthood is considerably reduced relative to the benefit of complex housing beginning at weaning.

3. 4. 2. 4. Experiment 2C

Although rats with P7 lesions showed significant recovery on this task relative to the P2 operates in Experiments 2A and 2B, they were still impaired relative to sham-operated controls, confirming a result that we have found previously (e.g., Kolb, 1987). Furthermore, as in our earlier study (Kolb & Stewart, 1995), we found a sex-related difference as females with P7 lesions were more impaired than males with similar lesions (Figure 3.5). Finally, experience facilitated the rate of acquisition.

A four factor ANOVA (Group X Experience X Sex X Trial Block) was performed across Trial Blocks 1-9, and found a significant main effect of lesion ($F(1,32)=5.5, p=.02$), sex ($F(1,32)=18.0, p<.0002$), and trial block ($F(8,256)=73.1, p<.0001$) but not of experience ($F(1,32)=0.2, p=.90$). There were two significant interactions: Lesion X Trial block ($F(8,256)=4.3, p<.001$) and Experience X Trial Block ($F(8,256)=2.3, p=.02$). The remaining interactions were small and non-significant, ($F's < 1, p's > .28$).

The two significant interactions are instructive. First, the Block X Lesion interaction reflected that the lesion animals learned the task more slowly than the control animals. Second, the Block X Experience interaction reflected that the animals housed in the complex environments learned the task more quickly than the lab-reared animals. There was, however, no differential effect of experience on the lesion animals as in Experiment 2A.

Finally, in order to determine if the animals had successfully learned the location of the hidden platform, we compared the performance of trial block 9, (the last set of trials to the original platform location), to trial block 10 (a set of four trials to a new location). A four way ANOVA (Group X Experience X Sex X Trial Block 9 and 10) found a significant main effect of the comparison of trial blocks 9 and 10 ($F(1,32)=50.8, p<.0001$) but all other effects were non-significant ($F's<1.5, p>.25$). Thus, all groups showed a significant disruption in performance from trial block 9 to 10, indicating that they knew the original location of the platform.

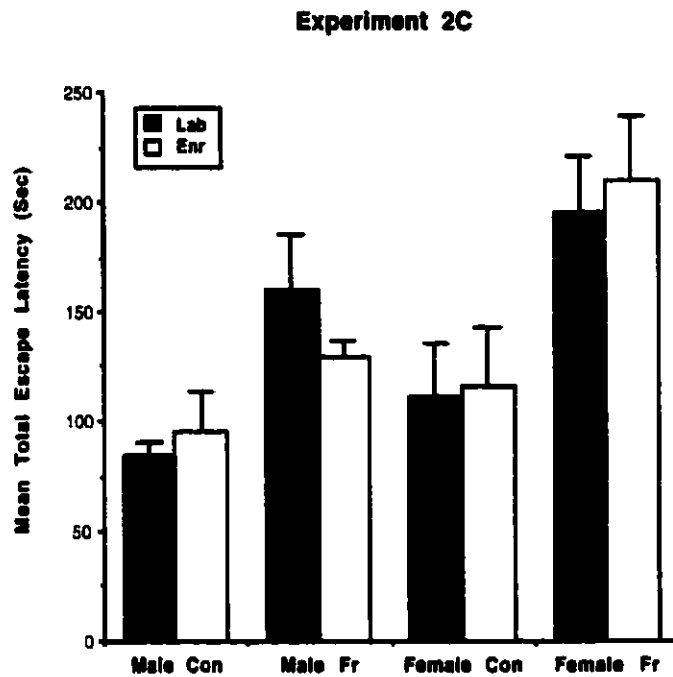


Figure 3.5. Water maze performance of P7 frontals and controls expressed as mean total escape latency. Complex rearing at weaning did not affect the performance of the P7 operates.

3. 4. 3. Skilled Reaching

3. 4. 3. 1. Experiment 2C

Control rats acquire the reaching task quickly, reaching asymptotic performance around 60% accuracy. As summarized in Figure 3.6 rats with frontal lesions in infancy do very poorly at this task with the males performing around 8% and the females around 30% accuracy. The females, but not the males showed a beneficial effect of the complex housing on reaching performance.

A three factor ANOVA (Group X Experience X Sex) revealed main effects of lesion group ($F(1,33)=114, p<.0001$), sex ($F(1,33)=14.2, p=.001$), but not of experience ($F(1,33)=1.5, p=0.24$). In addition, the Sex X Experience interaction was significant ($F(1,33)=7.1, p=.01$) but none of the other interactions were significant, F 's $<1.5, p$'s $<.2$). The Sex X Experience interaction reflects the obvious sex difference in the effect of experience shown in Figure 3.5: Females benefited from experience whereas males did not.

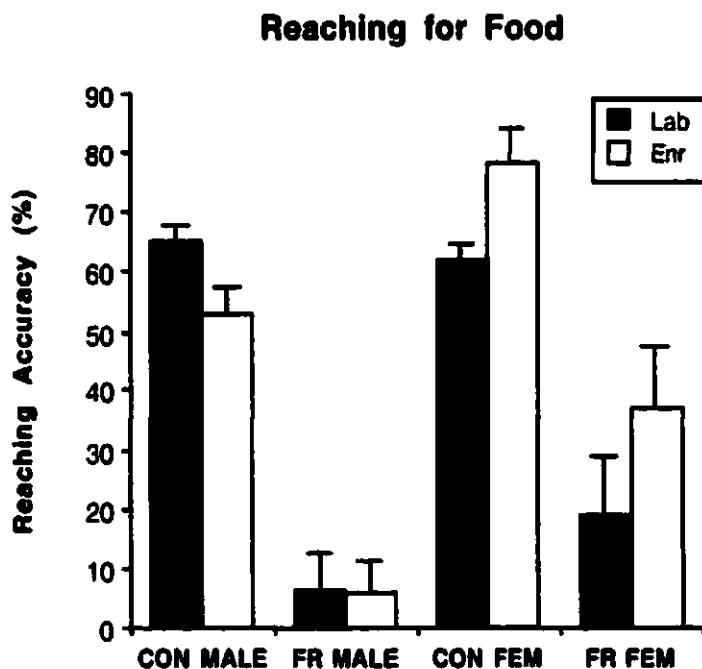


Figure 3.6. Performance of controls and P7 lesion animals on the Whishaw reaching task. Complex housing improved the performance of female animals but offered no benefit for males.

3. 5. ANATOMICAL RESULTS

3. 5. 1. General Observations

Gross inspection of the lesions in all three experiments showed that the lesions were roughly as intended with removal of Zilles' areas IL, Cg1, Cg3, anterior Cg 2, Fr2, and the medial portions of Fr1 and FL (Figure 3.7). There were no differences in lesion size between the lab- and enriched-frontal rats in any of the experiments, nor were there any obvious sex differences. There were, however, differences across the experiments. The lesions in Experiment 2A and 2B were slightly larger than those in Experiment 2C, as they included more damage to other adjacent motor areas, particularly the posterior parts of Fr1 and FL.

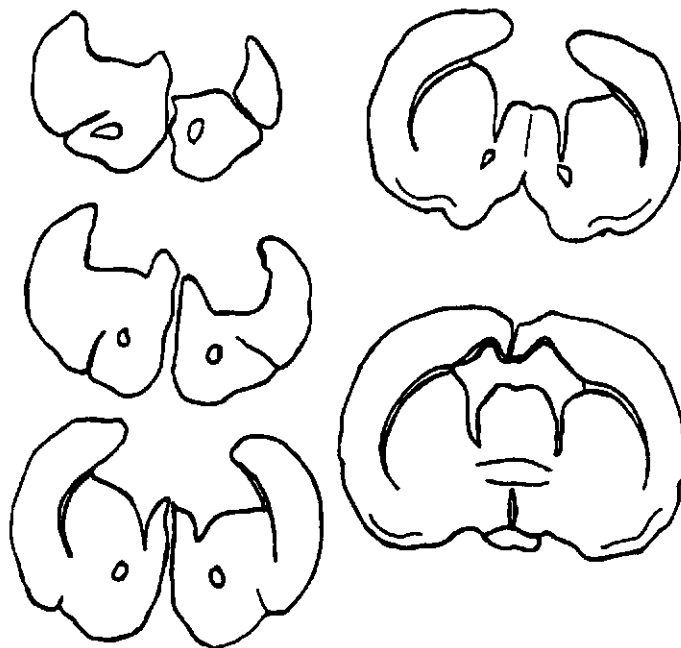


Figure 3. 7. Schematic illustration of a typical lesion from a P2 frontal lesion rat.

3. 5. 2. Brain Weight and Body Weight

One consistent result of our studies is that enriched housing produces reliable increases in brain weight of about 3-5%, which appear to be independent of body weight (Tables 3.1-3.3). Thus, in all 3 experiments, there was an increase in brain weight in enriched rats, whether or not they had frontal lesions.

Table 3. 1. Summary of brain weights for the rats in Exp 2A

Group	Experience		
	Cage-Housed	Complex-Housed	
<hr/>			
Female			
Control	1.89±.02	1.95±.03	(3%)
Frontal	1.47±.03	1.56±.02	(6%)
Male			
Control	2.01±.05	2.06±.05	(3%)
Frontal	1.55±.08	1.67±.01	(8%)

Numbers refer to means ± standard errors in grams.

Percentages in brackets indicates relative increase in brain weight in comparable complex-housed groups.

Table 3. 2. Summary of body weights for the rats in Exp 2A

Group	Experience		
	Cage-Housed	Complex-Housed	
<hr/>			
Female			
Control	268	239 (11%)	
Frontal	257	241 (6%)	
Male			
Control	448	358 (21%)	
Frontal	405	297 (27%)	

Numbers refer to means \pm standard errors in grams.

Percentages in brackets indicates relative decrease in body weight in complex- housed groups.

Table 3. 3. Summary of brain weights for the male rats in Experiments 2B and 2C

Group	Experience		
	Cage-Housed	Complex-Housed	
<hr/>			
Experiment 2B			
Control	2.18 \pm .01	2.24 \pm .02 (3%)	
Frontal	1.90 \pm .02	1.95 \pm .02 (3%)	
Experiment 2C			
Control	2.16 \pm .01	2.32 \pm .02 (7%)	
Frontal	1.94 \pm .04	2.06 \pm .02 (6%)	

Numbers refer to means \pm standard errors in grams.

Percentages in brackets indicate relative increase in brain weight in comparable complex-housed groups.

In Experiment 2A, there were main effects of lesion, experience, and sex ($F's > 6$, $p's < .02$) and there were no significant interactions (see Table 3.1). The effect of experience on brain weight in Experiment 2A contrasts to the effect of experience on body weight, which was in the opposite direction (see Table 3.2). ANOVA showed all main effects to be significant, $F's > 4$, $p's < .05$) and, in addition, there was a significant Sex X Lesion interaction, reflecting a lesion effect on body weight in males but not in females, a result that we have found on several previous occasions (e.g., Kolb, 1987).

Brain weights were only recorded for male rats in Experiment 2B (Table 3.3). As in Experiment 2A, there were significant main effects of lesion ($F(1,13)=140$, $p < .0001$) and experience ($F(1,13)=5.1$, $p < .04$) but not the interaction ($F(1,13)=0.10$, $P=.75$). Body weights were not recorded in Experiment 2B.

For Experiment 2C, there again were significant main effects for all factors ($F's > 49$, $p's > .0001$) but there were no interactions ($F's < 1.3$, $p's < .26$). Similarly, ANOVA on body weights showed a significant effect of sex ($F(1,29)=209$, $p < .0001$) but not of lesion nor experience ($F's < 2$). In addition, there was a significant Lesion X Housing interaction ($F(1,29)=6$, $p < .03$), which reflected the fact that the frontal animals lost relatively more weight in the complex environments than did the control animals.

In sum, the analysis of brain weight data indicates that housing animals in the complex environments resulted in increased brain size and decreased overall body size regardless of whether animals had frontal lesions or not.

3. 5. 3. Dendritic arborization and spine density

3. 5. 3. 1. Experiment 2B

There were three principal results of the dendritic analyses. First, P2 frontal lesions led to a reduction in branching and in spine density. Second, enrichment in adulthood increased both dendritic arborization and spine density. Third, the enrichment effects were larger in the lesion than in the control animals (Figure 3.8. & 3.9.). There were no sex differences so data were analyzed by collapsing across sex.

Two-way ANOVAs (Lesion X Experience) on the dendritic length on the apical and basilar fields, respectively, showed main effects of lesion ($F(1,58)=13.6, p<.0005$; $F(1,58)=4.5, p<.04$), which reflects the dendritic stunting in the lesion animals, experience ($F(1,58)=10.1, p<.002$; $F(1,58)=29.2, p=.0001$), which reflects the stimulatory effect of experience, and the Lesion X Experience interactions ($F(1,58)=4.0, p<.05$; $F(1,58)=7.6, p<.008$). The interactions reflect the relatively larger effect of experience on the lesion animals.

Two-way ANOVAs (Lesion X Experience) on the spine density on the apical and basilar fields, respectively, showed main effects of lesion

($F(1,58)=5.9, p<.02$; $F(1,58)=14.0, p<.0005$), experience ($F(1,58)=22.6, p<.0001$; $F(1,58)=24.3, p<.0001$), but neither interaction was significant ($p's>.3$). Thus, the lesion led to a decrease in spine density whereas experience stimulated an increase in spine density.

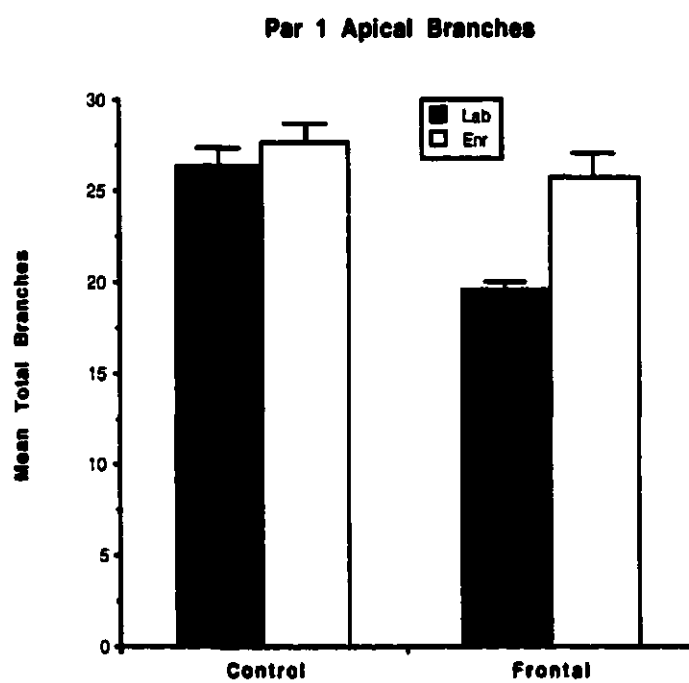


Figure 3.8. Summary of apical dendritic branching of control and P2 lesion animals following complex rearing in adulthood. Complex rearing restored some of the dendritic material lost as a result of the lesion.

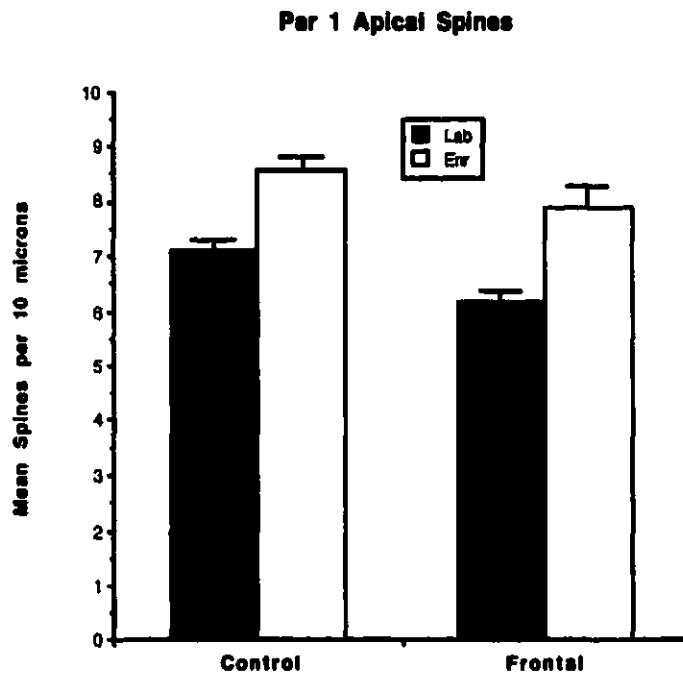


Figure 3.9. Spine density in control and P2 frontal animals following complex housing in adulthood. The lesion decreases spine density whereas exposure to complex housing increases spine density.

3. 5. 3. 2. Experiment 2C

Because the effects of lesion and experience were qualitatively different in the dendritic length and spine density measures, we consider them separately.

3. 5. 3. 2. 1. Dendritic length

There were three overall effects. First, there was a lesion effect on dendritic length as frontal animals showed a significant atrophy in dendritic length in both the parietal and occipital cortex (Figure 3.10). Second,

experience increased dendritic length in all groups. Third, there were sex differences in the effects of experience in occipital cortex of the control groups with males showing a greater increase than females.

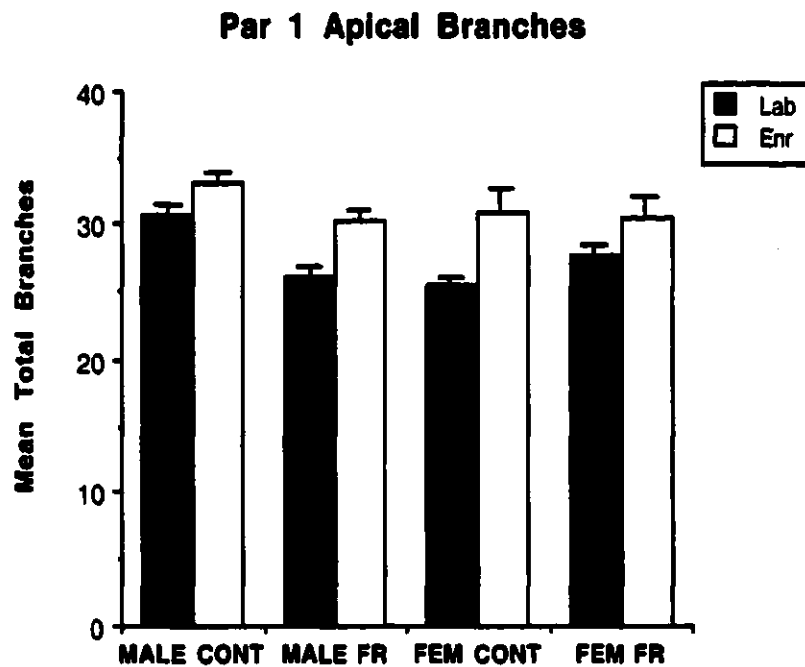


Figure 3.10. Effect of lesion and experience on total number of apical branches in area Par 1.

For the apical field of parietal cortex there were main effects of lesion ($F(1,64)=5.1, p<.03$), experience ($F(1,64)=17.4, p<.0001$), but not sex ($F(1,64)=0.001, p=.98$). Only the Experience X Sex interaction approached significance ($F(1,64)=3.6, p=.06$), reflecting the slightly smaller effect of experience in males than in females. ANOVA on the basilar field found a

significant effect of lesion ($F(1,64)=15.5, p<.0002$), but not of experience or sex ($p's>.5$). The only significant interaction was Experience X Sex ($F(1,64)=4.3, p=.04$), which reflected an effect of experience on the male, but not the female, rats.

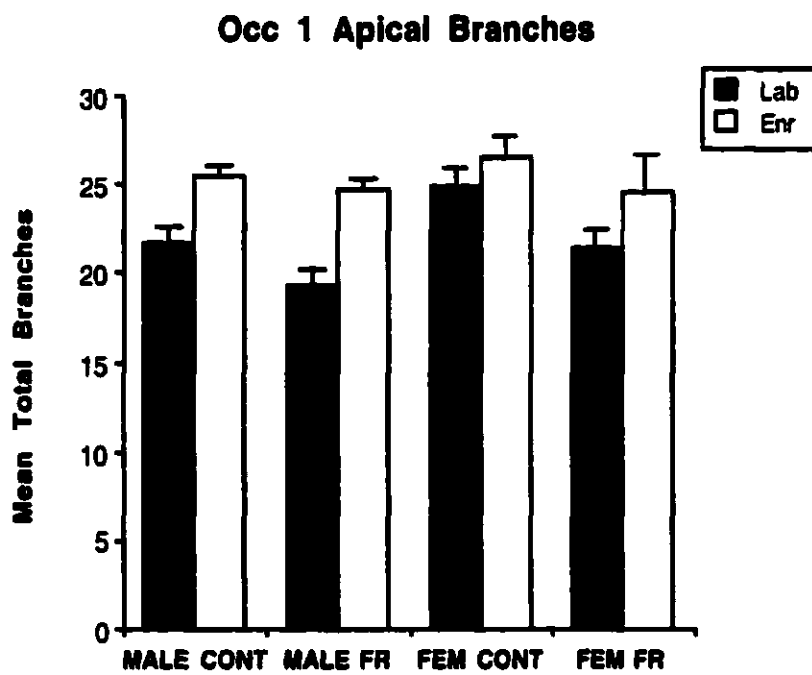


Figure 3.11. Effect of lesion, experience, and sex on the total branch number in area Occ 1.

Analysis of the apical fields of the occipital cortex found a main effect of lesion ($F(1,64)=27.8, p<.0001$), experience ($F(1,64)=17.2, p<.0001$), and sex ($F(1,64)=3.7, p=.05$). All of the interactions were significant ($p<.05$ or better) except for the Lesion X Experience interaction ($p=.97$). The three-way

interaction reflected the fact that only the control females failed to show an effect of experience but, curiously, the control females raised in lab cages had significantly more dendritic arbor than any of the other groups. Finally, ANOVA on the basilar field of the occipital cortex showed main effects of lesion ($F(1,64)=20$, $p<.0001$), experience ($F(1,64)=11.5$, $p<.001$) but not sex ($p=.11$). There was again a significant three-way interaction ($F(1,64)=3.9$, $p<.05$) as well as an Experience X Sex interaction ($F(1,64)=4.3$, $p<.05$). These interactions reflected the finding that there was no effect of experience in the female controls.

3. 5. 3. 2. 2. Spine Density

Again, there were two overall results. First, there was an increase in spine density after frontal lesions in male, but not female, animals (Figure 3.12). Second, animals given complex housing beginning at weaning showed decreases in spine density relative to lab animals. This result is the opposite of that in Experiment 2B in which we found increased spine density in animals given complex housing beginning in adulthood (Figure 3.9) but consistent with the results of Experiment 1. Both males and females showed this effect overall, although it was much smaller in the females, especially the lesion females. Separate ANOVAs (Lesion X Experience X Sex) were performed on the apical and basilar measures for each of the parietal and occipital areas, respectively.

Analysis of the apical tips for the parietal cortex revealed a significant main effect of experience ($F(1,61)=10.6, p<.01$), sex ($F(1,61)=10.6, p<.01$), and the three way interaction ($F(1,61)=4.8, p<.01$). The three-way interaction reflected the increased spine density in the frontal males, but not females, as well as the decreased spine density in response to experience. None of the other F values approached significance ($p's>.23$). A similar analysis on the basilar tips found a main effect of experience ($F(1,61)=16.9, p<.01$), the Lesion X Experience interaction ($F(1,61)=10.4, p<.002$), as well as the three way interaction ($F(1,61)=6.0, p<.02$). The interactions reflected the fact that there was a significant increase in spine density in the frontal males and a decrease in spine density in response to experience, a result that was only significant in the lesion animals. None of the other F values approached significance ($p's>.24$)

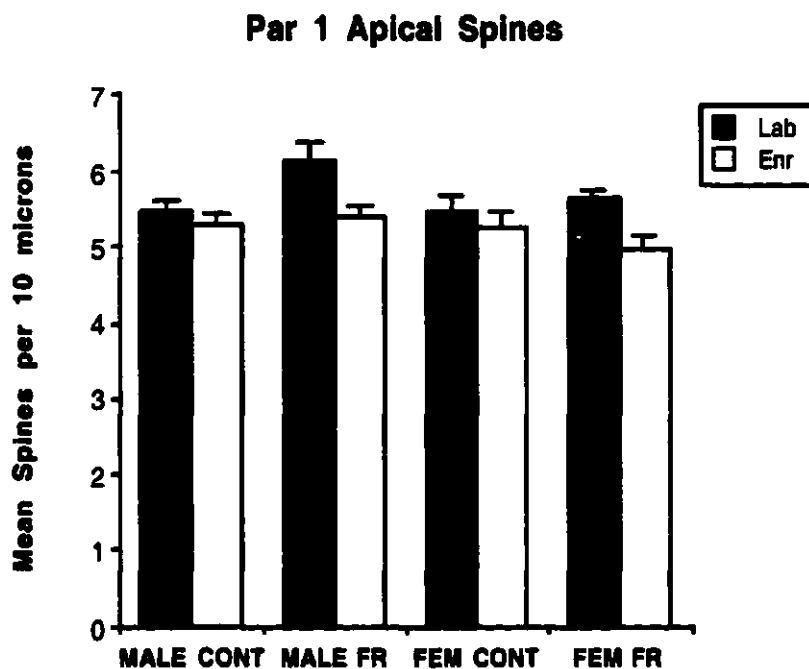


Figure 3.12. The effect of P7 lesion was to increase spine density in area Par 1 in male animals but there was no lesion-related effect in female animals. Complex housing reduced spine density across all groups.

Analysis of the occipital cortex showed a similar pattern. ANOVA on the apical tip found a main effect of experience ($F(1,61)=11.1, p<.001$), sex ($F(1,61)=6.8, p<.01$), and the Experience X Sex interaction ($F(1,61)=4.6, p<.04$). The sex difference and Experience X Sex interaction reflected the generally higher spine density in males and the absence of any experience effect on the spine density in females (Figure 3.13). None of the other F values approached significance ($p's>.20$). Finally, ANOVA on the basilar tip found a main effect of experience ($F(1,61)=7.5, p<.01$) and the Lesion X Sex interaction ($F(1,61)=6.3,$

$p < .01$). The interaction reflected that finding that whereas males with frontal lesions showed an increase in spine density, females showed a significant decrease. Again, none of the other F values approached significance (p 's $> .13$).

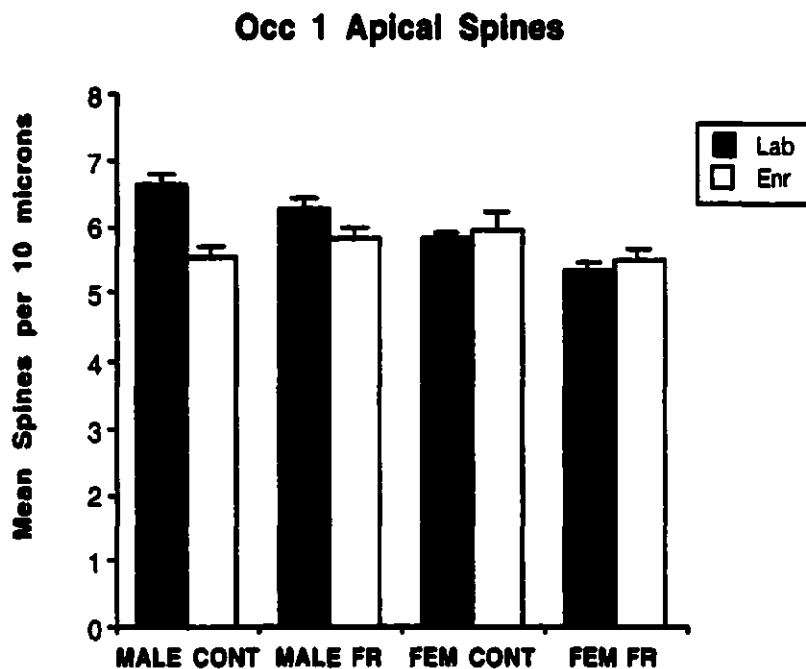


Figure 3.13. Complex rearing decreased spine density in area Occ 1 in male animals but had no effect in females.

3.6. DISCUSSION

There were six principal findings of these studies. First, housing in complex environments increases the dendritic arborization of both the normal and the infant-damaged brain. Second, the effects of experience on spine density vary with age that the experience is begun. Third, frontal

cortical lesions in the first days of life severely disturb behavioral development. In contrast, frontal lesions on postnatal day seven produce less severe deficits in spatial learning than perinatal lesions. These lesions do produce, however, severe deficits in skilled motor performance. Fourth, rearing animals with perinatal lesions in complex environments reduces the effects of perinatal frontal lesions but the earlier the experience begins, the better the outcome. Fifth, rearing animals with P7 lesions in complex environments, even when begun at weaning, does little to further stimulate functional recovery. Sixth, the effects of experience on both behavior and neuronal morphology are sexually dimorphic in rats with P7 frontal lesions.

3. 6. 1. Complex housing increases dendritic arborization.

The current study replicates findings shown previously by many investigators who have shown that housing animals in various types of complex environments increases dendritic arborization (e.g., Coleman & Riesen, 1968; Rosenzweig & Bennett, 1978; Sirevaag & Greenough, 1988; Walsh, 1982). Furthermore, the current study replicates the finding of Juraska and her colleagues (e.g., Juraska, 1990) that there is a basic sex difference in the effect of complex housing, at least in visual cortex. Thus, whereas males show an increase in dendritic arborization, females show virtually no effect at all. We did find a significant increase in parietal cortex, however, suggesting that there may be some fundamental difference between the effects of experience on the parietal and occipital cortex, at least in females.

3. 6. 2. The effects of experience on spine density are age-dependent.

Although it is generally believed that complex housing leads to increased spine density there are actually very few studies showing this effect (e.g., Globus et al., 1973). Our current result replicates our earlier finding that spine density is increased when animals are placed in complex environments as adults but spine density is decreased when animals are placed in the same environment as weanlings (Experiment 1). It thus appears that similar experiences can have qualitatively different effects at different ages. In particular, it appears that sensory and/or motor stimulation has unexpected effects on the young brain. In fact, it is not just the weanling brain that shows these effects. In particular, we have found that newborn rats that are tactilely stimulated daily with a small paintbrush over the first two weeks of life also show a decrease in spine density when the brains are examined in adulthood (Kolb, Gorny, & Gibb, 1994).

Our finding of a decrease in spine density in young animals is not without precedent (e.g., Bock & Braun, 1998; Wallhauser & Scheich, 1987). For example, Wallhauser and Scheich (1987) presented newly hatched chicks with a hen or an acoustic stimulus with the goal of imprinting the chicks to the visual or auditory stimulus. They found that neurons in the hyperstriatum of the imprinted chicks showed a *decrease* in spine density in the trained chicks when the brains were examined 7 days after training. There is an important caveat to this result, however. Patel, Rose, and Stewart

(1988) used a Golgi technique to impregnate chick brains 25 hours after training chicks to avoid a colored bead. They found a twofold *increase* in spine density in the neurons in a region of the hyperstriatum in the “trained” chicks. The critical difference between the Wallhausser and Scheich study and the Patel and colleagues’ study is the survival time. Thus, taken together the experiments show that there was an increase 25 hours after training but there was a decrease seven days after training. The simplest conclusion from the chick studies is that the novel stimulation may cause an initial rapid increase in spine density, followed by a pruning. If we extrapolate to the current study we might predict that the juvenile animals showed an increase in spine density over the first hours or days in the condominiums, followed by a synaptic pruning.

At this point we do not know what the drop in spine density in the young animals actually reflects. It is possible that the early experience also stimulates changes in glial or neuronal proliferation. In the latter case, it could therefore be that there are more neurons but each neuron has fewer connections. This remains to be proven, however.

3. 6. 3. The effects of cortical lesions in infancy are age-dependent

We have shown in a long series of experiments that cortical lesions in the first few postnatal days have more severe behavioral effects than those around 5-7 days (e.g., Kolb, 1987; 1995). Recovery is task dependent, however, as the best functional outcome can be seen in tests of cognitive ability whereas

performance on tests of motor or species typical behavior is minimal (e.g., Kolb & Whishaw, 1981). The results of the current experiments are consistent with this previous work. Animals with frontal lesions on day two were more severely impaired on the water task than animals with lesions on day seven. These P7 animals were severely impaired, however, on a test of skilled reaching. It appears that cognitive behaviors show much better functional recovery after cortical injury at days 7-10 than do species typical or motor behaviors.

We should note here that a comparison of the effects of day two lesions in Experiments 2A and 2B shows that the animals in Experiment 2A had a more severe impairment in spatial learning. This difference is likely due to the change in the details of the behavioral procedures in the two experiments. Although the lesions in Experiment 2B were slightly smaller, we have shown elsewhere that lesion size in P2 animals is not related to the extent of impairment in the water task (Kolb & Cioe, 2000).

3. 6. 4. Experience can attenuate the effect of early cortical injury but earlier is better.

We have shown previously that rearing rats in complex environments beginning at weaning stimulates recovery in rats with frontal lesions on postnatal days one or five (Kolb & Elliott, 1987). In contrast, placing rats with adult frontal lesions in similar environments is without functional benefit (Kolb & Gibb, 1991). The comparison of the effects of Experiments 2A and 2B

suggest that complex housing is beneficial to rats with infant cortical lesions but the effect of experience is greatest if it begins early in life. The brains of the animals placed in complex environments either in infancy or adulthood both are altered by the experience, but apparently the experience has a greater effect on recovery if it occurs while the brain is still developing. This result has important implications for the design of therapies for brain-injured children. Indeed, in a parallel series of studies we have shown that tactile stimulation during the first week after frontal cortical injury in the first few days of life stimulates even greater functional recovery than seen in the current studies (e.g., Kolb, Gorny & Gibb, 1994).

3. 6. 5. There may be limits to cortical plasticity after early cortical lesions.

Rats with frontal lesions on postnatal day 7-10 show much reduced functional deficits relative to rats with similar lesions earlier in life. This functional recovery is correlated with an increase in spine density after P7 lesions (Kolb, Stewart, & Sutherland, 1997; current study) and an increase in both dendritic length and spine density after P10 lesions (Kolb & Gibb, 1992). Experiment 2C showed that rats with P7 frontal lesions showed only a small behavioral and anatomical effect of complex experience. It appears that there are limits to cortical plasticity after early injury. On the other hand, it is clear that the P7 brain did change with experience. In particular, experience produced a reversal of the increase in spine density resulting from the lesion. This result is counterintuitive as one might predict that if the increased spine

density was supporting recovery, then reversing this increase would interfere with recovery. Indeed, this is the case in animals with noradrenaline depletion (Kolb, Stewart & Sutherland, 1997). One possible explanation is that the experience is not only altering spine density but it may be affecting other morphological events such as glial or neural proliferation. This remains to be shown, however.

3. 6. 6. The behavioral and anatomical effects of P7 frontal lesions are sexually dimorphic.

The results of the current study support and extend our previous results showing that P7 frontal cortical lesions have sexually dimorphic effects on both behavior and neuronal morphology (Kolb & Stewart, 1995; Kolb, Petrie, & Cioe, 1998). In our earlier studies we found that male rats with P7 frontal lesions showed better performance on spatial learning tasks than did female rats with similar lesions. The current study showed that although this was once again true, females appeared to show better recovery on a test of skilled motor behavior. We did not find any evidence of a sex difference in behavioral outcome after P2 lesions, however, as neither sex show much recovery on skilled reaching.

Similarly, there are sex differences in anatomical response to early injury. As shown previously (Kolb & Stewart, 1995) males show an increase in spine density that is not observed in females. This increased spine density may play some role in facilitating recovery on measures of cognitive

behavior, such as the spatial learning measure in the current study, but it evidently does not facilitate recovery of skilled motor behavior. At this point we do not know what morphological changes might correlate with the improved motor skill performance in the females.

4. EXPERIMENT 3: TACTILE STIMULATION AFTER CORTICAL INJURY

4.1. ABSTRACT

Rats with bilateral lesions (and sham controls) of the medial frontal or posterior parietal cortex were treated with tactile stimulation for 15 min three times daily for two weeks following perinatal decortication. In adulthood they were trained in a spatial navigation task and a skilled reaching task, their brains were removed, and dendritic length and spine density were analyzed for layer III pyramidal neurons. Tactile stimulation significantly reduced the behavioral impairments after early cortical injury. Stimulation had different effects in sham and cortically-injured rats: There was an experience dependent decrease in spine density in sham animals but no spine-density change in lesion animals, who also showed an increase in dendritic length relative to untreated operates. These results suggest that early intervention after cortical injury may be important for stimulating plastic changes in the cortex that can underlie functional recovery.

4.2. INTRODUCTION

Perinatal cortical injury has severe behavioral and anatomical sequelae in both laboratory animals and human infants. For example, rats with frontal or posterior parietal lesions on the first days of life have more severe behavioral deficits than animals with similar injuries in adulthood. Furthermore, this poor behavioral outcome is associated with a thin cortical mantle and a general atrophy of dendritic fields in remaining cortical pyramidal cells (for reviews see Kolb, 1990; 1995). Similarly, human children with cerebral injuries in the third trimester, a time that is roughly embryologically equivalent to newborn rats, are at high risk for severe behavioral disturbances (e.g., Stiles et al., 1988; Vargha-Khadem, O'Gorman & Watters, 1985). We therefore asked if there might be behavioral treatments that could attenuate the devastating functional consequences of early brain injuries. Because it had been shown that tactile stimulation is effective in stimulating growth in premature infants (Field, Schanberg, Scafidi, et al., 1986) and newborn rats (Schanberg & Field, 1987) we decided to evaluate the effect of tactile stimulation on recovery from cortical injury in newborn rats.

4.3. MATERIALS AND METHODS

4.3.1. Subjects

The study was done with 148 rats from 14 litters of animals. Rat pups sustained a frontal lesion, parietal lesion, or sham surgery on postnatal day

two (P2) or four (P4). Half of the litters then received tactile stimulation for two weeks beginning on the day following surgery. This yielded 24 non-stimulated controls, 28 stimulated controls, 18 non-stimulated parietals, 20 stimulated parietals, 25 non-stimulated frontals, and 33 stimulated frontals. There were approximately equal numbers of males and females in each group. The tactile stimulation consisted of sequentially stroking individual pups with a soft camel hair paint brush for 15 minutes 3X per day.

4. 3. 2. Surgical Procedures

The animals were anesthetized on P2 (frontal lesions and littermate sham controls) or P4 (parietal lesions and littermate sham controls) by cooling them in a Thermanon cooling chamber until their rectal body temperature was 18-20° Celsius. The rats receiving frontal cortex removals first had their frontal bone excised with iris scissors and then the underlying cortex removed by gentle aspiration. The cortex intended for removal included Zilles' (1985) regions Cg1, Cg3, and PL as well as the medial portion of Fr2 of the motor cortex. The animals receiving posterior parietal cortex removals underwent a similar surgical procedure wherein the presumptive posterior parietal cortex area was determined by dividing the distance between the bregma and the lambda into thirds. The skull over the middle third was removed with iris scissors from 1 mm lateral to the sagittal fissure to a point equal to anterior-posterior length and then the underlying cortex was gently aspirated taking special care not to open the ventricle. In this case the cortex

intended for removal consisted of the anterior portion of Zilles' areas OC2MM and OC2ML. As soon as the removal of cortex was complete the animals were sutured with fine silk thread. The sham-operated animals were anesthetized in a similar manner and then the scalp was incised and then sutured. Control animals were identified by removing the outer toe on the right hind paw.

4. 3. 3. Stimulation procedure

The pups in the stimulated groups were removed from their mother and placed in a Plexiglas cage that had a 1 cm deep layer of "bed of cobs" on the bottom. The pups were transported to an adjacent room and were given gentle tactile stimulation with a 0.5 cm diameter camel hair histology brush for 15 min three times daily (9 AM; 1 PM; 4 PM). They were then returned to their mother, having been away from her for no more than 20 min. The stimulation procedure continued for 14 consecutive days. During the first week of stimulation the animals typically went into REM sleep, as characterized by twitching. By the time the animals reached about 14 days old they had become quite active and the experimenter had to follow the animals with the paintbrush as they explored the holding cage in order to provide the stimulation.

4. 3. 4. Behavioral tasks

4. 3. 4. 1. Morris water task

The method used in this test was similar to the procedure described by Sutherland et al. (1983) and is based on the original task described by Morris (1981). The maze was a circular pool (1.5 m diameter X 0.5 m deep) with smooth white walls. The pool was filled with approximately 25° C water and mixed with 500 ml of skim milk powder to render the water opaque. A clear Plexiglas platform (11 X12 cm) was placed in a constant position inside the pool approximately 12 cm from the wall. The water level was adjusted so that the platform stood 2 cm below the surface of the water. The platform was invisible to a viewer outside the pool and to a rat swimming in the water. A trial consisted of placing a rat into the water at one of four start locations (north, south, east, or west) around the pool's perimeter. Within a block of four trials each rat started at the four locations in a random sequence, and each rat was tested for four trials a day over five consecutive days. If on a particular trial a rat found the platform, it was allowed to remain on it for 10 seconds. A trial was terminated if the rat failed to find the platform after 90 seconds. Each rat was returned to its holding cage for approximately five minutes before the next trial commenced. The swimming path for each rat was tracked by a computer tracking system in the frontal study and by an experimenter tracing the swim path by hand in the parietal study. The

former analysis allowed a calculation of swim distance. The latter analysis was restricted to a measure of whether the animals deviated from an imaginary corridor (one rat length wide) that ran from the start location to the platform. Rats in the frontal experiment were trained for two trial blocks per day for eight consecutive days. Rats in the parietal experiment were trained for only five days.

4.3.4.2. Skilled reaching

The reaching procedure, developed by Whishaw, Pellis, Gorny, and Pellis (1991) was used to assess the skilled forelimb movements of each rat after being trained to reach for chicken feed pellets in Plexiglas cages (28 cm deep x 20 cm wide x 25 cm high). The front and floor of each cage were constructed with 2 mm bars separated from each other by 1 cm, edge to edge. A tray (5 cm deep x 2 cm wide x 1 cm high) containing chicken feed pellets, was mounted in front of each cage. To obtain food, the rats had to extend the forelimb through the bars, grasp, and retract the food pellet. The food tray was mounted on runners to adjust the distance of the food from the bars. Distance adjustments ensured that each rat could not simply rake the food into the cage. Bars on the floor ensured that if the rat dropped the pellet it would irretrievably lose it and would have to reach again. Rats were trained on the task for a maximum of three weeks before video taping. During the first week, the rats were grouped in pairs in the reaching cages for one hour a day to allow the rats to adapt to their new surroundings. The food

deprivation schedule commenced during the first week, and each rat was provided with 15 grams of laboratory rodent food daily following the training period. The rats were subsequently trained individually for one hour each day during the second week whereas during the third week, this training period was shortened to 5–15 minutes a day. Five minutes of continuous reaching activity for each rat was videotaped and scored when the rats were approximately five months of age. If the rat made a reaching movement (forepaw inserted through the bars, but no food was grasped or it was dropped), it was scored as a "reach". Whereas if the rat obtained a piece of food and consumed it, the movement was scored as a "reach" and a "hit." Scoring was achieved by calculating the percentage of hits to total reaches for each animal's preferred forelimb. Left and right paw reaches and hits were recorded separately. Only the rats in the frontal experiment were trained on this task.

4. 3. 5. Anatomical methods

Following the completion of behavioral testing the animals were given an overdose of sodium pentobarbital and intracardially perfused with a solution of 0.9% saline. The brains were then removed from the skull and trimmed by cutting the olfactory bulbs approximately 5 mm ahead of the frontal edge of the cortex, and the optic nerves 2 mm ahead of the optic chiasm. The pineal body and paraflocculi were removed and the spinal cord cut even with the posterior edge of the cerebellum. The brains were weighed

and then immersed whole in 20 mls of Golgi-Cox solution. The brains were stored (in the dark) in the Golgi-Cox fixative for 14 days before being transferred to a solution of 30 % sucrose for two to seven days. The tissue was cut at 200 μm on a Vibratome™ then developed using a method described by Gibb and Kolb (1998).

Layer III pyramidal cells from Zilles' area Par 1 were traced using a camera lucida at 250X. In order to be included in the data analysis, the dendritic trees of the pyramidal cells had to meet the following criteria: (a) the cell had to be well impregnated and not obscured with blood vessels, astrocytes, or heavy clusters of dendrites from neighboring cells; (b) the apical and basilar arborizations had to appear to be largely intact and visible in the plane of section. Cells were chosen by locating the section at the level of the anterior commissure and then by drawing each cell in the section that met the criteria listed above. The cell drawings were then analyzed using two different procedures. In the first, each branch segment was counted and classified by branch order using the procedure of Coleman and Riesen (1968). Branch order was determined for the basilar dendrites such that branches originating at the cell body were first order; after one bifurcation, second order; and so on. In the second, based on a method described by Sholl (1956) an overlay of concentric rings was used to determine the number of dendritic crossings and ultimately an estimation of dendritic length.

Spine density was measured from one apical dendritic branch in the terminal tuft, one secondary apical branch beginning about 50% of the

distance between the terminal tuft and the soma, one basilar terminal branch, and one secondary basilar branch. Spine density measures were made from a segment greater than 10 μm in length, and usually about 50 μm . The dendrite was traced (1000X) using a camera lucida and the exact length of the dendritic segment calculated by placing a thread along the drawing and then measuring the thread length. Spine density was expressed as the number of spines per 10 μm . No attempt was made to correct for spines hidden above or beneath the dendritic segment so the spine density values are likely to underestimate the actual density of the dendritic spines.

4. 4. BEHAVIORAL RESULTS

4. 4. 1. Morris water task

Injury to either the frontal or posterior parietal region produced a marked deficit in spatial learning performance relative to sham control animals and this deficit was significantly reduced by the tactile stimulation (Figure 4.1). In fact, the tactilely stimulated brain-injured animals showed such an improvement that many animals were able to perform nearly as well as controls. Because the frontal and parietal experiments were conducted two years apart and because there had been modifications to the testing room and procedure, it was decided to analyze data from the two experiments separately. Because we had found sex differences in previous studies of frontal lesions, we included sex as a factor in the frontal ANOVAs.

For the frontal experiment a three-way ANOVA with Lesion, Experience, and Sex as factors showed a significant main effect of lesion on total latency ($F(1,42)=11.2, p<.002$), but not of experience ($F(1,42)=2.3, p=.13$) nor sex ($F(1,42)=0.3, p=.60$). None of the interactions were significant ($p's>.14$). ANOVA on the swim distance showed a main effect of lesion on total swim distance ($F(1,42)=9.78, p<.005$), a marginal effect of experience ($F(1,42)=3.8, p<.06$), but not sex ($F(1,42)=0.9, p=.53$). In addition, the Lesion X Treatment interaction was significant ($F(1,42)=7.5, p<.01$) but none of the other interactions were significant ($p's<.36$). The interaction reflected the fact that experience only affected the performance of the frontal rats.

For the parietal experiment a two-way ANOVA with Lesion and Experience as factors showed a main effect of both lesion ($F(1,46)=11.5, p<.0001$) and experience ($F(1,46)=6.6, p=.01$), but there was no interaction ($F(1,46)=1.3, p=.27$). In the parietal experiment the swim paths were drawn by hand rather than by computer, so an ANOVA on the errors in swim paths was done in this study. A two-way ANOVA on total errors, with Lesion and Experience as factors showed a significant main effect of lesion ($F(1,46)=11.2, p<.002$) but not of experience ($F(1,46)=2.6, p=.11$) or the interaction ($F(1,46)=0.1, p=.72$). Thus, although there was a drop of about 20% in the number of errors for both the control and lesion group, the difference was not statistically reliable.

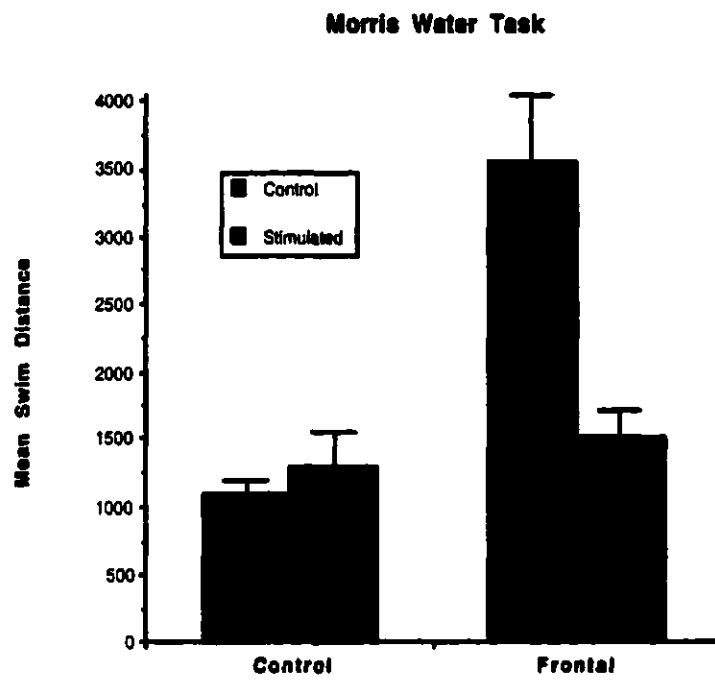


Figure 4.1. Performance of control and P4 frontal lesion animals on the Morris Water Task expressed as mean swim distance. Tactile stimulation markedly improved the performance of the P4 frontal animals.

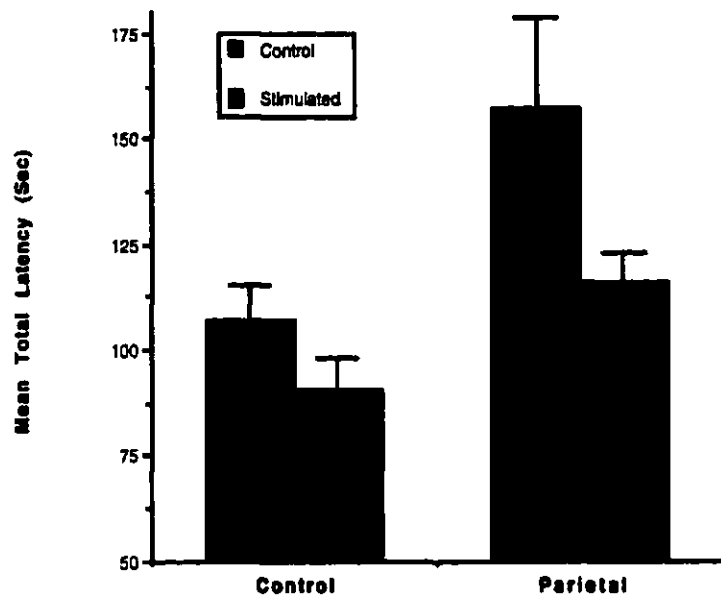


Figure 4.2. Performance of control and P4 parietal lesion animals expressed as total latency. Tactile stimulation improved performance of the lesion animals.

4. 4. 2. Skilled reaching

Rats with frontal lesions were impaired on the skilled reaching task and both control and frontal rats showed an improvement in reaching performance with tactile experience (Figure 4.2). A three- way ANOVA was performed with Lesion, Experience, and Sex as factors. There was a significant main effect of lesion ($F(1,68)=89.5, p<.0001$), experience ($F(1,68)=8.0, p<.0002$) and sex ($(F(1,68)=5.9, p<.02)$). None of the interactions were significant

($p > .48$). Posthoc tests showed that both the lesion and control animals benefited significantly from the early experience and that overall, females were better at this test than males.

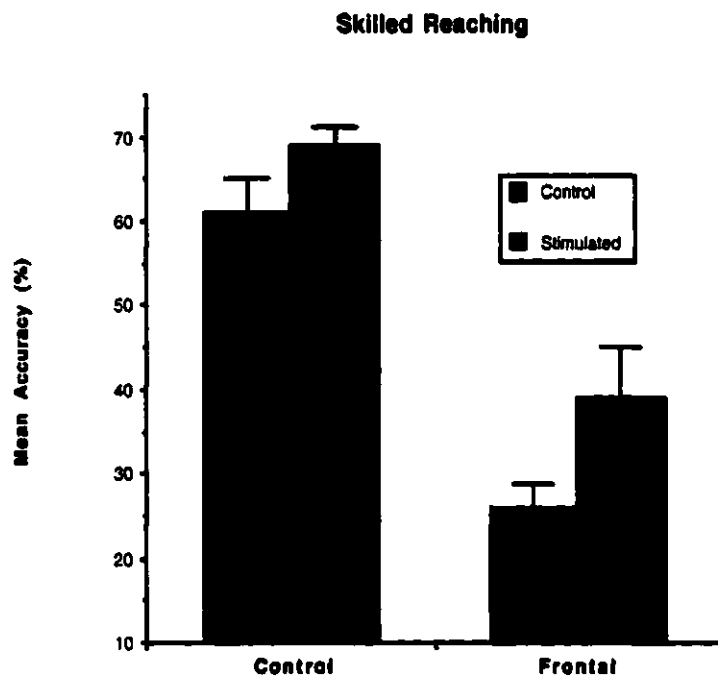


Figure 4.3. Skilled reaching performance of control and P4 frontal lesion animals expressed as % accuracy. Tactile stimulation improves performance of the lesion animals on this task.

4.5. ANATOMICAL RESULTS

4.5.1. General observations

Gross inspection of the lesion brains showed that the lesions in the frontal group were roughly as intended with removal of Zilles' areas Cg1, Cg3, and the medial portions of Fr2, as well as the more lateral portions of Fr2 in

some animals. There was no damage to the striatum or olfactory bulb. Similarly, the lesions in the parietal group were roughly as intended and included the posterior part of Zilles' area Par 1 and the anterior portion of Oc2, much as described in more detail elsewhere (Kolb & Cioe, 1999). There was no damage to the hippocampal formation but it was distorted in shape as it shifted forward to move partially into the lesion cavity. There were no differences in lesion size between the lab- and enriched-frontal rats in either lesion group, nor were there any obvious sex differences.

4. 5. 2. Brain weight

Brain weight was decreased by lesion and increased by tactile stimulation, the effect being about 3 % in each group (Table 4.1). For the frontal experiment a three- way ANOVA with Lesion, Experience, and Sex as factors revealed significant main effects for each factor ($F(1,96)=116.2, p<.0001$; $F(1,96)=3.92, p=.05$; $F(1,96)=33.9, p<.0001$). None of the interactions were significant ($p's>.30$). For the parietal experiment, in which the n's for the lesion groups was considerably smaller, especially when Sex is a factor, there was a main effect of lesion and sex ($F(1,43)=9.2, p<.005$; $F(1,43)=26.2, p<.0001$) but not of experience nor any of the interactions ($p's>.10$).

Table 4.1. Summary of Brain Weight of animals in Experiment 3

Group	Experience	
	No Treatment	Stroked
Male control	2.055 ± .027	2.080 ± .032
Male frontal	1.750 ± .035	1.812 ± .043
Male parietal	2.015 ± .027	1.945 ± .022
Female control	1.883 ± .036	1.919 ± .028
Female frontal	1.637 ± .028	1.699 ± .025

Numbers refer to means ± standard errors.

4. 5. 3. Dendritic length

There were three principal findings: 1) both parietal and frontal cortical lesions significantly reduced dendritic length; 2) tactile stimulation did not affect dendritic length in sham-operated animals; and, 3) tactile stimulation significantly reversed the loss in dendritic length in the basilar field of the parietal lesion animals (Figure 4.4). This latter effect reflected an increase of about 17% in total basilar dendritic length in the parietal animals, which is a very large increase relative to effects that we have observed in animals with other types of behavioral therapies after cortical injuries. There was no sex difference so the data were collapsed across sex to do Lesion X Experience ANOVAs.

ANOVA on the apical field revealed a significant main effect of lesion ($F(2,106)=8.9, p<0001$), but not of experience ($F(1,106)=0.3, p=.62$), nor the

interaction ($F(1,106)=0.2, p=.82$). Post hoc tests (Fisher's LSD, $p's <.05$) showed that the two lesion groups differed from the control group, but not from one another. ANOVA on the basilar field revealed a significant main effect of lesion ($F(2,106)=13.2, p<.0001$) but not of experience ($F(1,106)=1.1, p=.29$). The interaction also was significant ($F(2,106)=10.4, p<.0001$). The significant interaction reflected the fact that both lesion groups showed a significant drop in dendritic length in the non-stimulated groups but the parietal group showed a significant increase in dendritic length in the stimulated group, as illustrated in Figure 4.4 and 4.5.

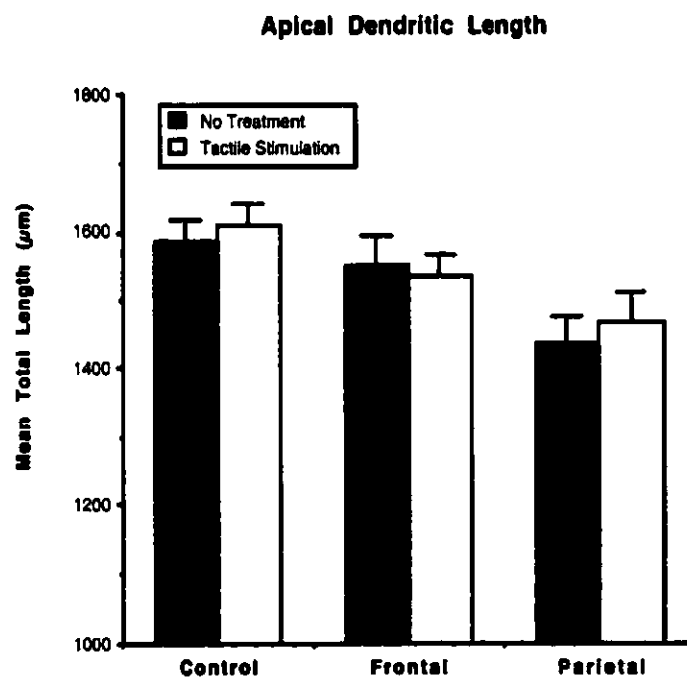


Figure 4.4. Effect of tactile stimulation on apical dendritic length in control, frontal and parietal lesion animals.

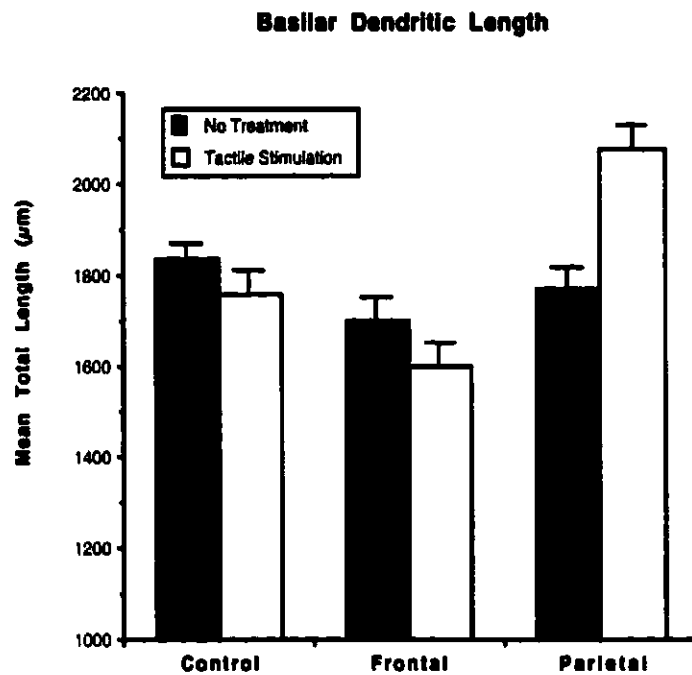


Figure 4.5. Effect of tactile stimulation on the length of basilar dendrites in control, frontal and parietal lesion animals.

4.5.4. Spine density

The analysis of dendritic spines led to three principal findings, as illustrated in Figure 4.6 and 4.7: 1) Rats with both frontal and parietal lesions, and did not receive tactile stimulation, showed a significant drop in spine density; 2) Sham-operated animals who received tactile stimulation had a significant *decline* in spine density in both the apical and basilar fields; and 3) The decline in spine density was not observed in either of the lesion groups, and was actually reversed in the frontal group. As there were no sex

differences, the data were collapsed across sex for ANOVAs with Lesion and Experience as factors.

ANOVA on the apical tips revealed a main effect of lesion ($F(2,220)=32.8, p<.0001$), experience ($F(1,220)=5.8, p<.02$), and the interaction ($F(2,220)=64.1, p<.0001$). Posthoc tests found that the lesion groups had significantly reduced spine density relative to the control group in the unstimulated group but for the stimulated animals the parietal group did not differ from control and the frontal group was significantly more dense than the control and parietal groups (p 's<.05 or better).

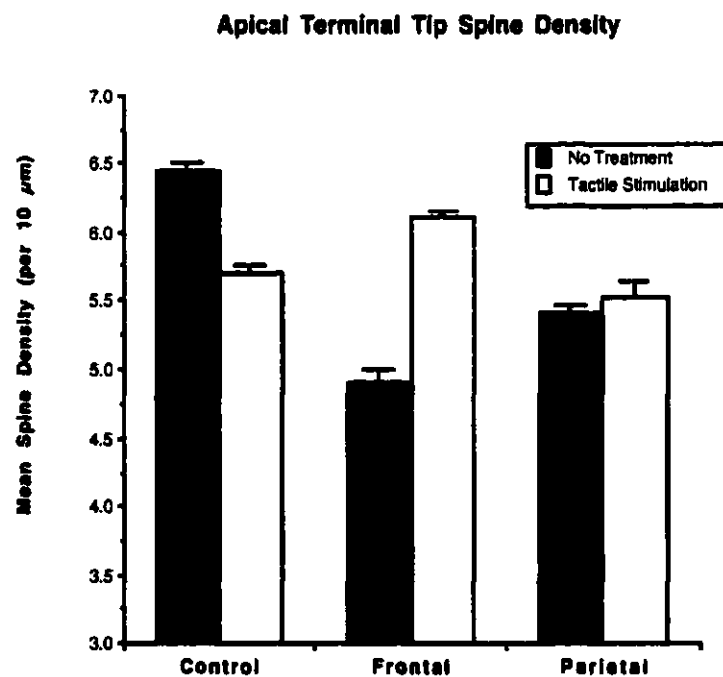


Figure 4.6. Effect of tactile stimulation on spine density in control, frontal and parietal lesion animals.

ANOVA on the basilar tips also found a main effect of lesion ($F(2,220)=11.6, p<.0001$) and the interaction ($F(2,220)=23.3, p<.0001$), but not of the main effect of experience ($F(1,220)=0.44, p=.51$). The posthoc tests again showed that although the lesion groups had a significantly lower spine density than control animals in the unstimulated condition, the frontal group had a significantly higher density than the controls in the stimulated condition and the control and parietal groups did not differ.

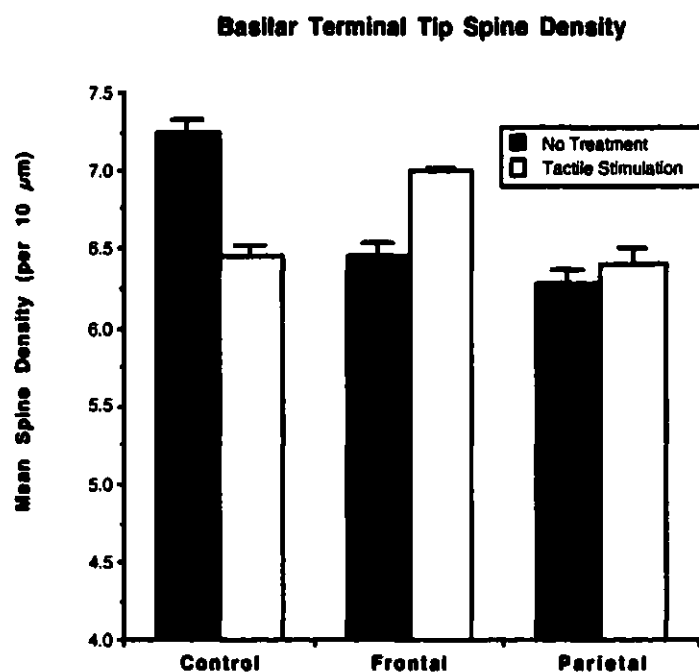


Figure 4.7. Effect of tactile stimulation on spine density in control, frontal and parietal lesion animals.

4. 6. DISCUSSION

Three novel findings resulted from this study. First, the tactile stimulation significantly reduced the behavioral impairments after both frontal and parietal lesions on P2 and P4, respectively. Second, the tactile stimulation for two weeks during infancy significantly decreased spine density in the adult brains of sham-operated control animals. Third, the tactile stimulation increased the dendritic length in the parietal animals and increased, rather than decreased, spine density in the frontal and parietal lesion groups. It is thus reasonable to hypothesize that one mechanism that may support the attenuated behavioral deficits in the lesion animals is a change in intrinsic cortical circuitry. It seems unlikely, however, that this is the only effect of the early experience. For example, the increase in brain weight that we observed in both the tactilely stimulated sham and lesion animals is unlikely to be due to changes in spine density. We have shown in parallel studies that tactile stimulation in normal animals produces changes in acetylcholinesterase and astrocytic density in the cortex (Kolb, Gorny & Gibb, 1994). These changes might be expected to be enhanced in animals with cortical injuries and perhaps potentiated by the tactile experience.

There is a rich behavioral literature showing that stressful experiences in infancy can permanently affect the brain and behavior of adult rats (Levine et al., 1967; Meaney et al. 1988b; 1991). The current study suggests that early experiences need not have only negative consequences, however. Early intervention after cerebral injury appears to have an important impact upon

both brain and behavioral development. The importance of early intervention after cortical injury cannot be underestimated. We have shown elsewhere (Experiment 2A) that complex-environment (condominiums) versus cage housing can stimulate recovery after early frontal lesions but only if the complex housing begins at weaning. Even four months of complex rearing has a minimal effect upon recovery from early frontal lesions if it does not begin until adulthood (Experiment 2B).

We would be remiss if we did not address the question of how much and what type of stimulation is optimal. We do not yet know how long the stimulation period must be to produce significant benefits after early injury but it seems likely that the critical period for this stimulation is early in development when the animals are relatively immobile during the stimulation. Indeed, we have noted that in the first week or so of stimulation the animals typically enter into a sleep pattern that is punctuated by twitching. (This suggests that the animals are in rapid eye movement sleep.) By two weeks of age the animals are much more interactive with the environment and begin to wander around the cage as they are stimulated. This would seem to be a very different type of experience than during the earlier period. We chose to use tactile stimulation in young rats both because this is a major form of communication between infant rats and their mother but also because the receptor apparatus for receiving tactile stimulation is more mature at birth in the rat than are the auditory and visual systems. It

remains to be seen if stimulation in these systems might also be effective in facilitating recovery.

Finally, we note that the tactile stimulation was equally effective in stimulating recovery in animals with frontal and posterior parietal lesions. In addition, we have preliminary evidence that tactile stimulation is also effective in stimulating motor recovery in rats with motor cortex lesions (A. Witt-Lajeunesse & B. Kolb, unpublished observations). Taken together, these results suggest that this treatment may be a powerful therapy for stimulating recovery from early cortical injury, although the different effects on neuronal morphology in the two lesion groups suggests that the actual mechanism may not be the same in animals with different lesions.

5. EXPERIMENT 4: PRENATAL VS POSTNATAL TACTILE STIMULATION

5. 1. ABSTRACT

Fourteen litters of animals were included in this study. Each litter contained animals that received frontal cortex lesions on postnatal day three (P3) and sham-operated controls. Three litters of pups were born to mothers that received tactile stimulation prior to and during the entire pregnancy term. Four litters of pups were postnatally tactilely- stimulated from day four until weaning and four litters of pups were simply removed from the nest from day four until weaning (postnatal handling). The remaining three litters of animals received no treatment either pre- or postnatally. Both prenatal and postnatal tactile stimulation improved functional recovery of lesion animals on the Morris water task. Prenatal stimulated lesion males also showed improved performance on the reaching task. Tactile stimulation both pre- and postnatally caused increased expression of acetylcholinesterase in the cerebral cortex. Postnatal handled animals were not different from untreated animals in their level of expression of this enzyme. All treatments changed the basal levels of urine corticosterone as compared to untreated animals. It appears that both prenatal and postnatal tactile stimulation are effective in improving functional outcome after early brain injury. These processes stimulate changes in expression of the acetylcholinesterase enzyme which may be partly responsible for increasing cortical plasticity. Alterations in corticosterone levels may also play a role in improving functional recovery.

5. 2. INTRODUCTION

The benefit of experience on the behavioral performance of animals on many tasks has been well documented (e.g., Rosenzweig, 1971; Kolb & Elliot, 1987; Kolb & Gibb, 1991). The mechanisms that underlie this improvement have not yet been defined, but the impact of environment on brain organization is nonetheless impressive. Although studies designed to assess the value of complex environment as a therapy for brain damage in adult animals have met with limited success, our work on the young brain-injured animals has been very encouraging. These studies have shown that environmental stimulation plays a major role in reorganizing and creating circuitry that supports a surprising degree of behavioral recovery following perinatal injury both when animals receive complex housing at weaning and when they receive tactile stimulation after an injury (Experiments 2A, 2C, 3). It is not clear, however, exactly what experience is doing to alter brain organization and behavior. Denenberg, Brumaghim, Haltmeyer, and Zarrow (1967) and Meaney, Mitchell, Aitken, et al. (1991) have shown that by simply removing rat pups from their mothers for fifteen minutes three times a day (a procedure termed "postnatal handling"), the lifetime levels of corticosterone was reduced in the "handled" animals. This reduction in the basal level of corticosterone improved the response of these animals to stress in adulthood and as a result allowed significant sparing of hippocampal granule neurons that normally die off in senescence. It appears that this

intervention in early life translates into successful retention of spatial abilities in old age. We wondered whether the animals that received postnatal tactile stimulation were also affected by the simple intervention of removal from their mothers, as this period of removal for tactile stimulation paralleled precisely the period Meaney et al. (1991) used to isolate the postnatally “handled” animals.

In a study by Kiyono, Seo, Shibagaki, and Inouye (1985) pregnant females were housed in complex environments for the duration of their pregnancy. The male offspring were tested as adults in the Hebb-Williams maze and surprisingly showed improved maze performance as a result of their prenatal experience. These results led us to wonder what the effect of *prenatal* tactile stimulation might be on behavioral recovery following early frontal cortical damage and how it might compare to postnatal tactile stimulation. We were also interested in comparing postnatal tactile stimulation with postnatal handling in order to determine if postnatal handling alone had beneficial effects on functional recovery.

In the current experiment, rats were given early frontal lesions on postnatal day three (P3) and experience in the form of tactile stimulation either prenatally or postnatally. A subset of animals received P3 frontal lesions and were simply removed from the nest (postnatal handling), or received no treatment. All animals underwent behavioral testing in adulthood and their brains were processed in Lana’s fixative for

acetylcholinesterase histochemistry and immunohistochemistry, or Golgi-Cox fixative for morphological analysis.

5.3. MATERIALS AND METHODS

5.3.1. Subjects

Fourteen litters of Long-Evans rats were included in this study (n=170). Each litter contained animals that sustained frontal cortex lesions at postnatal day three (P3) and sham-operated littermate controls. Three litters of pups were born to mothers that received tactile stimulation one week prior to impregnation and throughout the entire pregnancy term (7 male frontal, 6 male control, 12 female frontal, and 12 female control). Three litters of pups were born to mothers that received no stimulation during their pregnancy (10 male frontal, 10 male control, 6 female frontal, and 7 female control). After the pups were born no further stimulation was offered. The animals were not handled again until behavioral testing commenced at postnatal day 60 (P60). Of the eight remaining litters of pups born to mothers that received no stimulation during pregnancy, four litters were postnatally tactilely-stimulated (10 male frontal, 15 male control, 10 female frontal, 18 female control) and four litters were postnatally handled (10 male frontal, 11 male control, 12 female frontal, 14 female control).

5. 3. 2. Surgical procedures

The animals were anesthetized on postnatal day three (P3) by cooling them in a Thermoatron cooling chamber until their rectal body temperatures were in the range of 18-20°C. For the frontal cortex removals the frontal bone was removed by cutting it with iris scissors, and frontal decortication was achieved by gentle aspiration. The intent was to remove the medial subfields of the prefrontal cortex including the presumptive Zilles' (1985) regions Cg1, Cg3, and PL as well as the medial portion of Fr2 of the motor cortex. As soon as medial frontal decortication was achieved, the animals' scalps were sutured with silk thread. The sham-operated animals were anesthetized in the same manner, and the skin was incised and sutured. The sham-operates were identified by removing an outer toe on the right back foot.

5. 3. 3. Enrichment procedures

The mothers of pups that underwent prenatal tactile stimulation were stroked with a baby hairbrush and rubbed on their ventrum beginning one week before mating and continuing for the entire period of gestation. The stimulation lasted for 15 minutes three times daily (9AM; 1PM; 4PM). During this time the mother was offered food treats such as granola, crackers, peanuts, etc. Similarly, mothers of pups that received no prenatal stimulation were offered the same food treats in comparable quantities in their home cage.

Animals that received postnatal tactile stimulation were removed from the nest and individually stroked with a camel hair paintbrush for 15 minutes three times daily (9AM; 1PM; 4PM) beginning the day following surgery until weaning at postnatal day 21. Animals in the postnatally handled group were removed from the nest and placed in a Plexiglas cage in the same room where the postnatal tactile stimulation was conducted, for 15 minutes three times per day. Mothers of the postnatal treatment animals were offered food treats when the pups were removed from the nest and again when the pups were returned. The pups received no further stimulation after weaning.

5. 3. 4. BEHAVIORAL METHODS

5. 3. 4. 1. Morris water task

Beginning at P60 animals were trained on the Morris Water Task using a similar procedure to that described by Sutherland et al. (1983) based on the original task described by Morris (1981). The maze consisted of a circular pool (1.5 m diameter X 0.5 m deep) with smooth white walls. The pool was filled with approximately 25 °C water mixed with 500 ml of skim milk powder, used to render the water opaque. A clear plexiglas platform (11 X 12 cm) was placed in a constant position inside the pool approximately 30 cm from the pool wall. The water level was adjusted so that the platform was invisible to a viewer outside the pool and to a rat swimming in the water. A trial consisted of placing a rat into the water facing the pool edge at one of four

compass locations (north, south, east, or west) around the pool's perimeter. Within a block of four trials each rat started at the four locations in random sequence, and each rat was tested for four trials a day over five consecutive days. If on a particular trial a rat found the platform, it was permitted to remain on it for 10 seconds. A trial was terminated if the rat failed to find the platform after 90 seconds. Each rat was returned to its holding cage for approximately five minutes before the next trial commenced. The swim path for each rat on every trial was recorded using a Poly Track video tracking system (San Diego Instruments) which tracks the swim path and records the latency, distance and dwell time within each quadrant.

5.3.4.2. Skilled reaching

Following water maze training, animals were trained in a skilled reaching task developed by Whishaw, Pellis, Gorny, and Pellis (1991). In this task rats were trained to retrieve chicken feed through metal bars at the front of the Plexiglas training cage (28 cm deep x 20 cm wide x 25 cm high). The front and floor of each cage were constructed with 2 mm bars separated from each other by 1 cm, edge to edge. A tray (5 cm deep x 2 cm wide x 1 cm high) containing chicken feed pellets, was mounted in the front of each cage. To obtain food, the rats had to extend their forelimbs through the bars, grasp, and retract the food pellet. The food tray was mounted on runners to adjust the distance of the food from the bars. Distance adjustment ensured that each rat could not simply rake the food into the cage. Any pellets that the rat dropped

inside the cage were irretrievably lost through bars on the floor and the animal would have to reach again. During the first few days the rats were trained in pairs in the reaching cages for a period of one half hour per day. Once reach training commenced, the animals were provided with 15 grams of rat chow daily following the training period. The rats were subsequently trained individually for one half hour per day and then at the end of a two-week training period their performance was videotaped for a five-minute interval. Each time the rat reached through the bars whether or not food was obtained was scored as a "reach" and each time food was successfully returned to the cage and consumed was scored as a "hit". The percentage of hits to total reaches was then calculated for each animal's taped performance.

5.3.4.3. Circadian Activity

Following reach training the animals were placed in computer monitored circadian activity cages. These cages were designed to assess activity over a 24-hour period by monitoring motion in cages fitted with infrared light beams and detectors. Each time the animal disrupts the light beam the computer records the side of the cage at which the activity occurred and a combined activity (for activity occurring across both the left and right sides of the cage) was also computed. The animals occupied the activity cages for two consecutive 24-hour periods and their combined activity for the second day was analyzed. During activity monitoring the animals had ad lib access to both food and water and were maintained on a 12:12 hr light/dark

cycle. The time the animals were placed in the cages was noted and the subsequent analyses were matched for time of day. The animals in the prenatal stroked, control (no stroking) and postnatal stroked groups had urine samples collected within four hours of their initial placement in the activity cages. The urine samples then underwent radioimmunoassay for corticosterone levels using a procedure developed by Dean (2000).

5. 3. 5. ANATOMICAL METHODS

5. 3. 5. 1. Histological Procedures

At approximately 30 days of age a subset of the animals (n=65) were given an overdose of sodium pentobarbital and intracardially perfused with a solution of 0.9% saline in 0.1 M phosphate buffer (pH 7.2) followed by a solution of 4% paraformaldehyde and 11% picric acid in 0.1 M phosphate buffer (also known as Lana's Fixative). The brains were then removed from the skull and trimmed by cutting the olfactory bulbs approximately 5 mm ahead of the frontal edge of the cortex and the optic nerves 2 mm ahead of the optic chiasm. The pineal body and paraflocculi were removed and the spinal cord cut even with the posterior edge of the cerebellum. The trimmed brains were weighed and then postfixed in the Lana's solution at 4° C for 24 hours before cutting at 50 um on a Vibratome™. Five consecutive sets of tissue were saved and one was mounted immediately for acetylcholinesterase histochemistry. Three sets were saved for immunohistochemical staining and the final set was mounted and processed for Cresyl Violet staining.

At the conclusion of behavioral testing the remaining animals were given an overdose of sodium pentobarbital and intracardially perfused with a solution of 0.9% saline. The trimmed brains were weighed and then immersed whole in 20 mls of Golgi-Cox solution. The brains were then stored (in the dark) in the Golgi-Cox fixative for 14 days before being transferred to a solution of 30% sucrose for seven days. The tissue was cut at 200 μm on a Vibratome™ then developed using a method described by Gibb and Kolb (1998). The tissue processed for Golgi evaluation is currently undergoing analyses and will not be considered in the discussion of this work.

5. 3. 5. 2. Anatomical Analyses

Cortical thickness measurements were obtained from Cresyl Violet stained coronal sections projected on a Zeiss-Jena MF2 projector at a magnification of 20X (following the method described by Stewart & Kolb, 1988). Briefly, three cortical measures were made at points medial, central and lateral on five sections of tissue identified by the following landmarks; Plane 1: first caudate-putamen visible, Plane 2: anterior commissure, Plane 3: first hippocampal section, Plane 4: posterior commissure, Plane 5: last hippocampal section. A plastic metric ruler was used to measure from the edge of the cortex to the edge of the white matter. An average for each plane and for each animal was calculated and used for statistical comparison.

Lesion size was estimated for all animals in lesion groups from whole brain pictures taken with a digital camera. The digital images were opened in the Scion Image program then the lesion traced around its perimeter and the area analyzed. The same procedure was repeated for the whole brain (including the lesion area) and then the ratio of lesion/brain was calculated for an estimation of lesion size.

Acetylcholinesterase (AChE) staining was assessed using the Scion Image densitometry program. The tissue was placed on a Zeiss microscope using a 20X neofluor objective and the image captured on computer with a video camera. The same planes as were used for cortical thickness measurements were analyzed for AChE density. An average density was then computed for each animal and used for statistical comparison.

5. 4. BEHAVIORAL RESULTS

5. 4. 1. Morris water task

The control animals in all groups easily learned the location of the hidden platform and were soon able to find it quickly from all start sites. The P3 frontal animals in non-stroked litters were initially impaired at locating the platform and had resulting high sum latencies over the five days of testing (Figure 5.1). Although the control animals did not differ in their performance across trials as a result of any of the treatments, this was not true of the lesion animals. Animals that received pre- or postnatal tactile stimulation after P3 frontal removal were faster at locating the platform than

the non-stimulated lesion animals. Lesion animals that received postnatal handling showed no improvement in performance, however. The postnatally handled lesion animals were actually worse on the last trial block than the non-stimulated lesion group. (There were no sex differences in any of the groups on the water task and no interaction of Sex with Group, Treatment or Trial Block so the data were collapsed across sex.)

A three-way repeated measures ANOVA (Lesion X Treatment X Trial Block) revealed a main effect of lesion ($F(1, 93) = 46, p < .0001$), but no main effect of treatment ($F(3, 93) = 1.1, p = .3$) and no interaction of Treatment X Lesion ($F(3, 93) = .6, p = .6$). There was a significant effect of trial block ($F(4, 372) = 151, p < .0001$), which reflected faster latencies on later trial blocks as the animals began to learn the task. Although there was no significant interaction of Trial Block X Treatment, there was a significant interaction of Trial Block X Lesion ($F(4, 372) = 7.9, p < .0001$). This result showed that lesion animals took significantly longer to learn the task over the training week than did the control animals. There was also a significant interaction of Trial Block X Lesion X Treatment ($F(12, 372) = 4.6, p < .0001$); thus showing the P3 lesion animals that received pre- or postnatal stroking were faster at acquiring the task than the untreated or postnatally handled animals. (Posthoc analysis using Fisher's PLSD showed the prenatal and postnatal tactile stimulation groups differed significantly from untreated animals.)

MORRIS WATER TASK

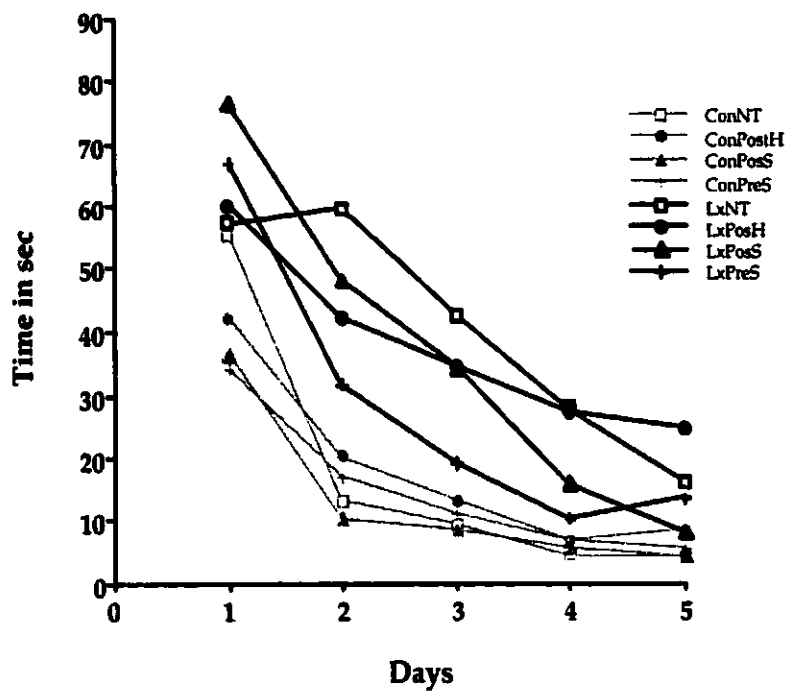


Figure 5.1. Acquisition curves for control and lesion treatment groups on the Morris Water Task. Among the lesion groups, the prenatally stroked animals show the fastest acquisition of the task.

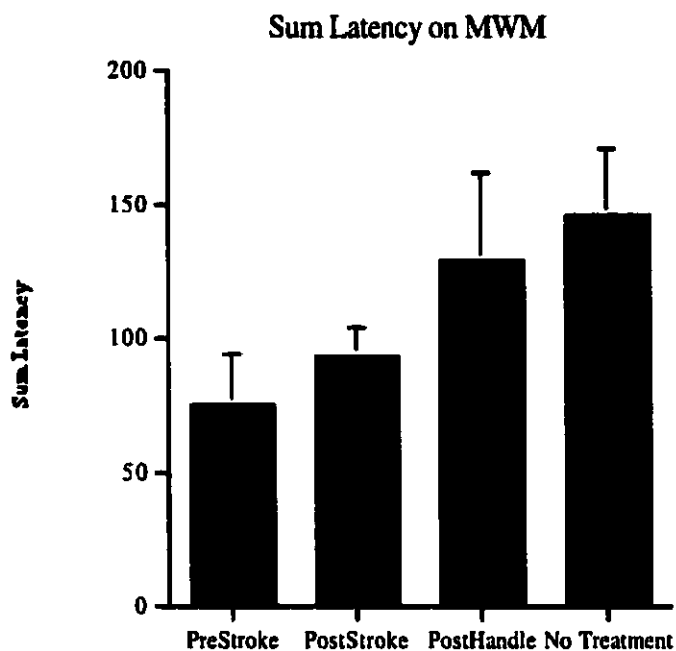


Figure 5.2. Water maze performance of P3 lesion animals expressed as sum latency. Prenatal and postnatal tactile stimulation significantly improved the performance of the P3 operates.

5.4.2. Skilled reaching

All animals with lesions, regardless of treatment, performed more poorly than sham-operated controls on this task and many of them failed to reach. Postnatal stroking had no effect on the performance of control animals but there was a non-significant trend for improved performance in lesion animals of both sexes (Figures 5.3 & 5.4). Prenatal stroking also had no effect on the reaching skill of control animals but there was an interesting effect on lesion animals. Although lesion female rats showed no benefit of prenatal stimulation, the lesion males were improved by the treatment. In

fact, the performance of the males in this group reached 42% accuracy whereas the untreated males were reaching at 24%. Perhaps the most puzzling result obtained from reaching assessment came from the postnatally handled animals. This treatment did have an effect on reach performance in control animals. In this case the males were unaffected but the females showed significant improvement (85% accuracy compared to 71% for untreated females) following this treatment. Postnatal handling did not improve the reaching accuracy of lesion animals of either sex. A three-way ANOVA (Lesion X Treatment X Sex) of the reaching data revealed a main effect of lesion on reaching performance ($F(1,75)=92.2, p<.0001$). There was no main effect of treatment nor sex on reaching, but there was an interaction of Sex X Treatment ($F(3,75)=3.4, p=.02$). This interaction was manifested by the divergent performance of male and female animals following treatment. Posthoc analysis using Fisher's PLSD showed that the postnatally handled group differed significantly from prenatal stroked animals ($p=.04$), postnatal stroked animals ($p=.02$), and untreated animals ($p=.004$)

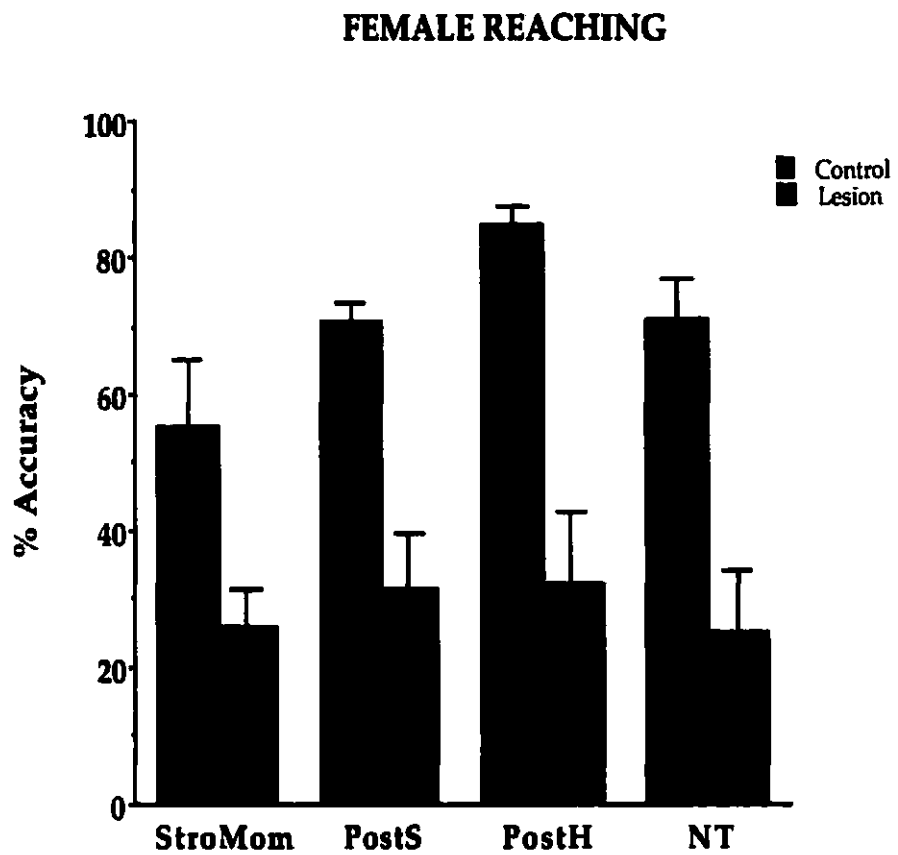


Figure 5.3. Reaching performance of female animals. Postnatally handled controls had a significantly better performance than controls from other treatment groups. (StroMom = prenatally stroked; PostS= postnatally stroked; PostH= postnatally handled; NT= no treatment)

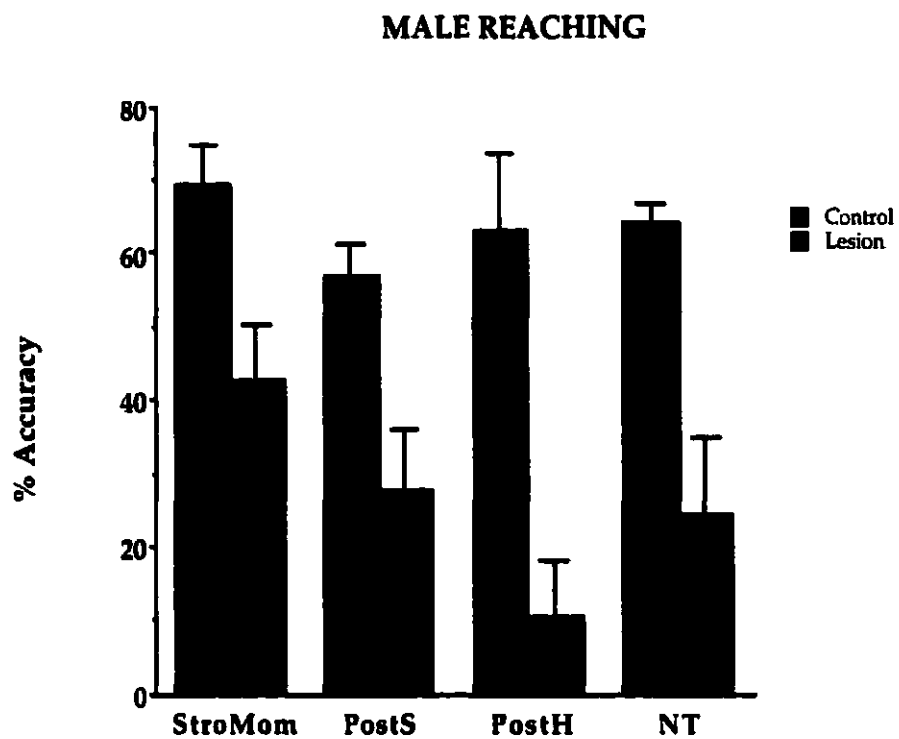


Figure 5.4. Reaching performance of male animals. Prenatally stroked lesion males show significant improvement in reaching performance over lesion animals from other groups

5. 4. 3. Circadian activity

Although there was no sex difference in activity levels in untreated control rats, lesion female rats were twice as active as lesion males. Prenatal stroking had no effect on the activity of either the sham-operated or lesion females but in males, prenatal stroking reduced the activity of the sham-

operated controls. Postnatal stroking and postnatal handling did not affect activity levels in sham-operates but postnatal handling reduced activity in both male and female frontal lesion animals. Postnatal stroking reduced activity in female frontal lesion animals but had no effect on the activity of frontal lesion males (Figure 5.5 & 5.6).

A three factor ANOVA (Lesion X Treatment X Sex) showed that there was no main effect lesion on circadian activity but there was a significant main effect of sex ($F(1,1760)=21.7, p<.0001$) and of treatment ($F(3,1760)=3.0, p=.03$). There was a significant interaction of Lesion X Treatment ($F(3,1760)=8.1, p<.0001$) reflecting that postnatal handling reduced activity in the P3 operates. There was also a significant interaction of Lesion X Sex ($F(1,1760)=11.5, p=.0007$) showing female operates and male controls were most active and Treatment X Sex ($F(3,1760)=2.8, p=.04$) indicating prenatal male controls and postnatally handled female operates had reduced activity compared to the other animals. There was no significant interaction of Lesion X Treatment X Sex. Posthoc analysis using Fisher's PLSD revealed that the treatment effect was due to the difference between the no treatment and postnatally handled groups ($p=.03$). The postnatally handled lesion animals showed much lower levels of activity than did lesion animals in the untreated group.

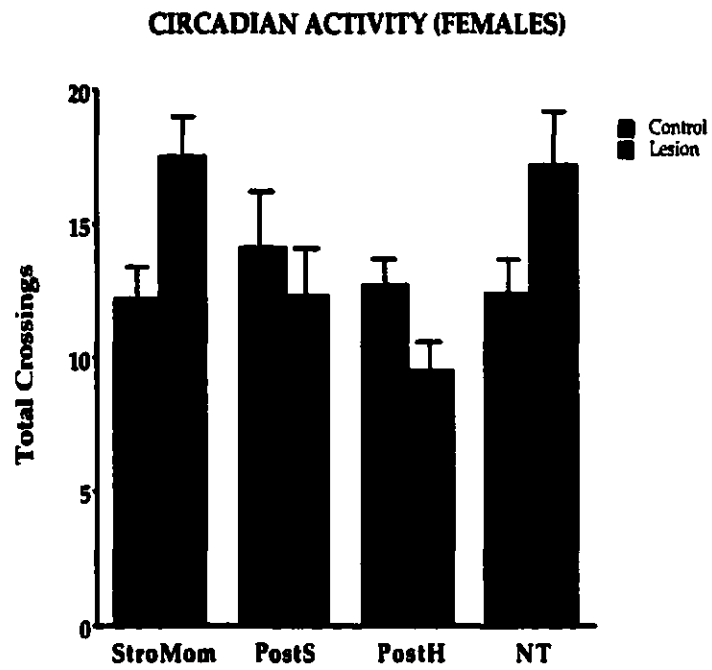


Figure 5.5. Combined activity for females summed across a 24- hour period. Postnatal stoking and postnatal handling reduced activity in lesion animals.

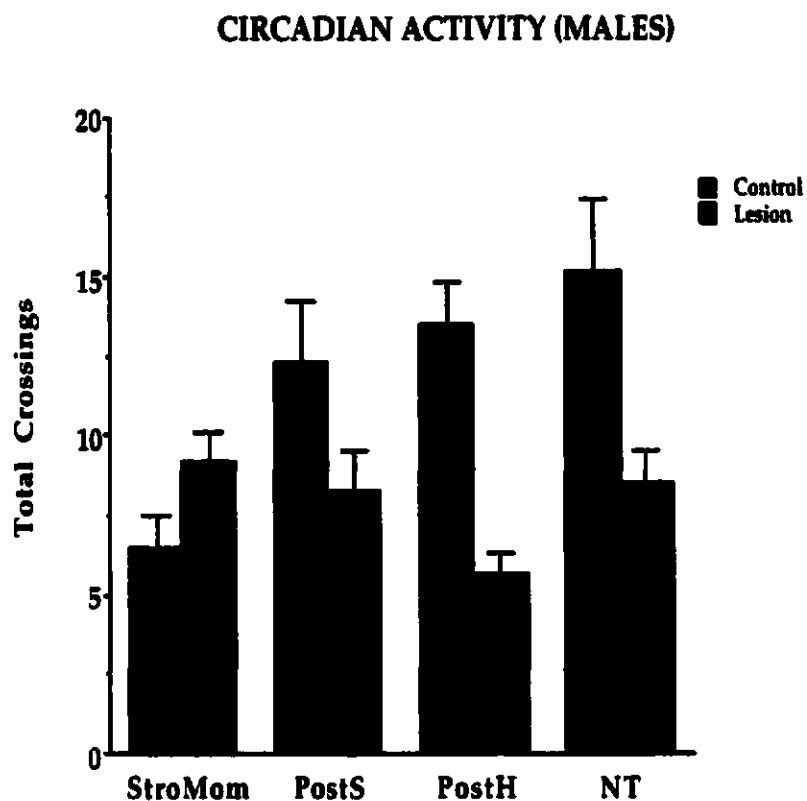


Figure 5.6. Combined activity for males summed across a 24- hour interval. Prenatal stroking reduced activity in control males whereas postnatal handling had a similar effect on lesion animals.

5.4.4. Urine Basal Corticosterone Levels

Urine analysis for corticosterone (CORT) was performed for five litters of animals. These animals were either prenatally or postnatally stroked, or untreated. The prenatally stroked males showed a reduction in basal CORT

whereas the prenatally stroked females showed an elevated response. In the postnatal tactilely-stimulated group both males and females showed a dramatic reduction in their basal corticosterone levels (Figure 5.7). Results are unavailable for the postnatal handled animals but in previous literature (Levine, Haltmeyer, Karas, & Denenberg, 1967; Meaney, Aitken, & Sapolsky, 1987) these animals show a characteristic reduction in basal urine corticosterone when compared to untreated animals. There was no effect of lesion on the basal corticosterone (CORT) levels in any of the treatments assessed so the data were collapsed across these groups. A two-way ANOVA (Treatment X Sex) revealed significant main effects of both treatment ($F(2, 43)=9.6, p=.0004$) and sex ($F(1,43), p=.009$). There was also a significant interaction of Treatment X Sex ($F(2,43)=4.4, p=.018$), which reflects the sexually dimorphic response of basal CORT after prenatal stroking.

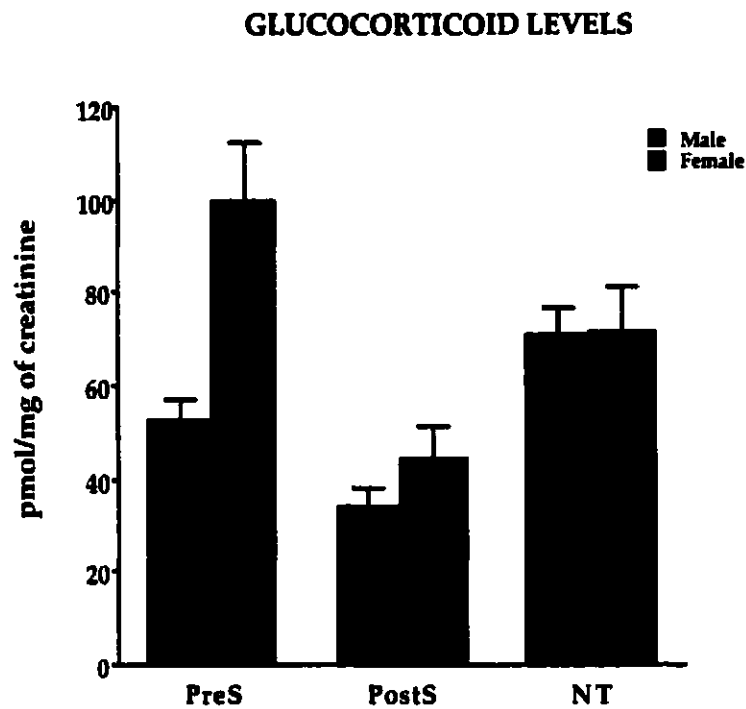


Figure 5.7. Basal glucocorticoid levels of prenatally stroked (PreS), postnatally stroked (PostS), and no treatment animals (NT) expressed as pmols of corticosterone per mg. of creatinine.

5. 5. ANATOMICAL RESULTS

5. 5. 1. Body weight

Because body weight is sexually dimorphic in rats and because there was an interaction between sex and treatment the results will be considered separately for the females and males. Female frontal lesion animals showed a

decline in body weight as compared to sham-operates but there was no further effect of treatment. A two-way ANOVA (Treatment X Lesion) showed there was no significant effect of treatment on the body size of female animals ($F(3,45)=1.1, p=.34$), but there was a significant effect of lesion ($F(1,45)=4.0, p=.05$). The interaction of Treatment X Lesion was also not significant ($F(3,45)=.32, p=.8$). The male animals showed a dramatic response to both variables, however. A two-way ANOVA (Treatment X Lesion) showed the lesion caused a significant drop in body weight across all groups ($F(1,38)=5.6, p=.02$). The treatment effect ($F(3,38)=4.3, p=.01$), was such that postnatally stroked animals showed the greatest increase in body weight and the postnatally handled animals the least weight gain over control values. Prenatally stimulated animals had intermediate values for body weight. The Lesion X Treatment interaction was non-significant ($F(3,38)=.633, p=.60$). Fisher's PLSD showed the prenatally stroked animals and postnatally stroked animals were significantly heavier than the untreated animals ($p=.03$ and $p=.002$, respectively). In the lesion animals the same relative effects of stroking were noted (i.e., increased body weight in both stroked groups relative to the untreated animals) but the handled animals showed a drop in body weight as compared to the untreated lesion males (Table 5.1 & 5.2)

Table 5.1. Summary of body weights for the female rats in Experiment 4

Experience	Group	
	Control	Frontal
Prenatal Tactile Stimulation	281.93 ± 14.96	271.86 ± 14.29
Postnatal Tactile Stimulation	295.96 ± 7.69	270.79 ± 12.33
Postnatal Handling	279.54 ± 9.28	271.38 ± 11.43
No Treatment	274.59 ± 18.05	247.40 ± 11.44

Numbers refer to means ± standard errors in grams

Table 5.2. Summary of body weights for the male rats in Experiment 4

Experience	Group	
	Control	Frontal
Prenatal Tactile Stimulation	423.84 ± 34.42*	389.99 ± 17.56*
Postnatal Tactile Stimulation	433.81 ± 8.78*	401.41 ± 18.64*
Postnatal Handling	400.28 ± 12.05	335.74 ± 6.38
No Treatment	362.64 ± .734	357.84 ± 22.43

Numbers refer to means ± standard errors in grams

*Differs significantly from untreated animals in same group

5. 4. 2. Brain weight

Brain weight is also sexually dimorphic in adult rats with males having larger brains than females, so for ease of comparison the male and female groups will be considered separately. As expected, the female lesion

animals showed reduced brain weight across all treatment groups when compared to their control counterparts. A two-way ANOVA (Treatment X Lesion) showed the lesion effect was significant ($F(1,45)=76.9, p<.0001$), but the stroking and handling treatments had no further effect on the brain weight in either control or lesion conditions. This was not the case in the male group, however. Again, a two factor ANOVA (Treatment X Lesion) revealed the effect of lesion was significant ($F(1,38)=79.6, p<.0001$), with the lesion animals having lighter brains. There was also a significant effect of treatment on brain weight ($F(3,38)=2.81, p=.05$). Posthoc analysis using Fisher's PLSD showed that control male animals had heavier brains after prenatal or postnatal tactile stimulation than did untreated or postnatally handled controls and in the case of the postnatally stroked animals this effect was significant ($p=.005$). In addition, the postnatal- handled lesion animals showed a further drop in brain weight relative to the no treatment lesion group (Table 5.3. & 5.4.).

Table 5.3. Summary of brain weights for the female animals in Exp 4

Experience	Group	
	Control	Frontal
Prenatal Tactile Stimulation	1.878 ± 0.021	1.718 ± 0.027
Postnatal Tactile Stimulation	1.920 ± 0.025	1.641 ± 0.032
Postnatal Handling	1.951 ± 0.012	1.647 ± 0.069
No Treatment	1.900 ± 0.053	1.652 ± 0.053

Numbers refer to means ± standard errors in grams

Table 5.4. Summary of brain weights for the male animals in Exp 4

Experience	Group	
	Control	Frontal
Prenatal Tactile Stimulation	2.030 ± 0.027	1.744 ± 0.041
Postnatal Tactile Stimulation	2.097 ± 0.037*	1.736 ± 0.031
Postnatal Handling	2.007 ± 0.047	1.577 ± 0.066*
No Treatment	1.960 ± 0.010	1.743 ± 0.054

Numbers refer to means ± standard errors in grams

*Differs significantly from untreated animals in same group

5. 4. 3. Cortical thickness

As with brain and body weight there is a sex difference in cortical thickness so males and females were analyzed separately. A two factor ANOVA (Lesion X Treatment) on the data collected from the female animals showed there was a significant main effect of lesion ($F(1,66)=107.3, p<.0001$) which reflected a thinner cortical mantle in the lesion animals. There was also a marginally significant main effect of treatment ($F(3,66)=2.5, p=.06$). Prenatal stroking reduced cortical thickness in control and lesion animals and posthoc analysis using Fisher's PLSD showed the prenatal stroked group

differed significantly from all other groups (p 's<.035). In the male animals the effect of lesion was the same as that noted in the females such that following lesion the cortical mantle is thinner than in sham-operated controls. A two factor ANOVA (Lesion X Treatment) revealed significant main effects for both lesion ($F(1,56)=30.6, p<.0001$) and treatment ($F(3,56)=4.2, p=.009$) but the interaction was non-significant ($F(3,56)=1.27, p=.29$). The treatment effect was such that postnatal stroking reduced cortical thickness in both control and frontal lesion animals (Table 3). Posthoc analysis using Fisher's PLSD revealed that the postnatally stroked animals had significantly thinner cortices than untreated animals ($p=.005$). Prenatal stroking and postnatal handling increased cortical thickness in the frontal lesion animals but the effect was not statistically reliable (Tables 5.5 & 5.6).

Table 5.5. Summary of cortical thickness for females in Experiment 4 (Magnification 20X)

Experience	Group	
	Control	Frontal
Prenatal Tactile Stimulation	51.29 ± 0.640*	43.43 ± 1.416*
Postnatal Tactile Stimulation	52.76 ± 0.432	45.74 ± 1.022
Postnatal Handling	52.55 ± 1.306	46.93 ± 0.406
No Treatment	52.55 ± 0.704	44.87 ± 0.517

Numbers refer to means ± standard errors in mm

* Differs significantly from all other animals in all treatment groups

Table 5.6. Summary of cortical thickness for males in Experiment 4.
(Magnification 20X)

Experience	Group	
	Control	Frontal
Prenatal Tactile Stimulation	52.26 ± 0.741	46.56 ± 0.881
Postnatal Tactile Stimulation	48.71 ± 1.153*	37.44 ± 1.386*
Postnatal Handling	50.80 ± 0.997	46.02 ± 0.357
No Treatment	52.25 ± 1.698	42.37 ± 2.411

Numbers refer to means ± standard errors in mm

* Differs significantly from animals in untreated group

5. 5. 4. Lesion size

The lesions were approximately as intended and were similar in size to the frontal removals in Experiment 2A (Figure 5.8), as they included damage to other adjacent motor areas (Fr1). The size of lesion (as estimated by dorsal surface area calculations) varied from 13.2% for the postnatally handled animals to 17.7% for the no treatment group (Figure 5.9). The prenatal and postnatal stroked groups had intermediate values (15% & 16.9%). A one-way ANOVA (Treatment) on lesion size showed no significant differences among the treatment groups ($F(3,34)=1.00, p=.40$).



Figure 5.8. Illustration of a typical lesion cavity found in P3 frontal lesion animals.

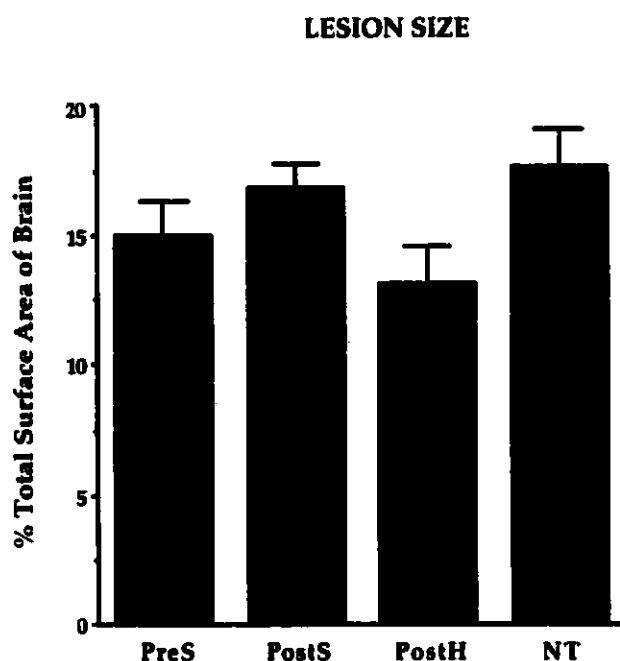


Figure 5.9. Lesion cavity size expressed as % total surface area of the brain. There were no significant differences in lesion size across treatment groups.

5. 5. 5. Acetylcholinesterase Density

Acetylcholinesterase (AChE) staining relies on enzyme activity to produce coloration that can be quantified. Variations in the composition of the reaction mixture and activity of the AChE enzyme can produce variation in the intensity of the stain. It is necessary to include appropriate controls in every batch of stain as it is not possible to directly compare densities between batches. Accordingly, statistical analysis was performed on tissues stained

within a batch. A three-way repeated measures ANOVA (Treatment X Lesion X Plane) revealed that prenatally stroked control animals had significantly higher values for AchE density than did the untreated controls ($F(1,12)=28.3$, $p=.0002$). There was no main effect of lesion on AchE staining but there was an interaction of Treatment X Lesion ($F(1,12)=80.3$, $p=.0001$). The effect of the lesion in the prenatally stroked animals was to reduce the amount of AchE staining whereas in the untreated group the lesion elevated the AchE staining (but not to the same degree as did the prenatal tactile stimulation in control animals). In a separate batch of AchE stained tissue, a comparison was made within the prenatal treatment group for sex differences and lesion effects. ANOVA showed there was no main effect of sex ($F(1,22)=2.0$, $p=.17$), but there was a main effect of lesion ($F(1,22)=32.1$, $p<.0001$), and a marginally significant interaction of Sex X Lesion ($F(1,22)=3.9$, $p=.06$). This interaction reflected a higher intensity of staining in control males over control females. In both male and female groups the effect of the lesion was to lower the intensity of the AchE stain with the net result being the lesion male and female animals had the same values for density of AchE staining.

Postnatal stroking increased the density of AchE staining as compared to non stimulated animals (Table 5.6.). A two-way ANOVA (Treatment X Sex) revealed that the treatment effect was significant ($F(1,40)=13.8$, $p=.0006$), but there was no effect of sex on the staining intensity ($F(1,40)=.2$, $p=.68$). A separate analysis on postnatally stroked animals looked at the intensity of AchE staining at postnatal day 10 and 17 in lesion and sham-operated

controls. At postnatal day 10 the tactile stimulation increased staining in both the control and lesion animals over control values but by postnatal day 17 stroking only increased Ache density in control animals. The untreated P3 operates showed an increase in AchE staining over untreated controls whereas postnatally stroked lesion animals showed a decline.

There was no difference in AchE staining between the postnatally handled and untreated groups. A two-way ANOVA (Treatment and Sex) showed no main effect of handling ($F(1,14)=1, p=.33$) and no sex effect ($F(1, 14)=.9, p=.35$). The interaction was also non-significant ($F(1,14)=.2, p=.7$). Within the postnatally handled group the lesion reduced the intensity of AchE staining but the effect was marginal ($F(1,18)=3.8, p=.07$).

Table 5.7. Relative Density of Acetylcholinesterase Staining

Experience	Group	
	Control	Frontal
Prenatal Tactile Stimulation	121.5*	109.8**
Postnatal Tactile Stimulation	123.6*	106.2**
Postnatal Handling	100.5	96.7
No Treatment	100.0	115.2**

Numbers refer to density expressed as % of untreated control values

* Differs significantly from animals in no treatment group

**Differs significantly from controls in same experience treatment group

5. 6. DISCUSSION

There were five principal findings from this experiment. First, prenatal stroking is as effective as postnatal stroking in improving behavioral recovery after early brain damage. Second, the effects of prenatal stroking appear to be sexually dimorphic. Third, postnatal handling does not facilitate behavioral recovery after early brain damage and may actually reduce functional improvement. Fourth, all treatments (prenatal stroking, postnatal stroking, and postnatal handling) change lifetime basal corticosterone levels. Fifth, both pre- and postnatal stroking treatments increase acetylcholinesterase expression within the brain (Figure 5.10). Each result will be considered in turn.

EXPERIMENT 4: CONTROLS

BEHAVIOR	Water Maze		Reaching		Circadian Activity		Corticosterone	
	M	F	M	F	M	F	M	F
SEX								
Pre Stroke	—	—	↑	—		—	↓↓	↑↑
Post Stroke	—	—	—	—	—	—	↓↓	↓↓
Post Handle	—	—	—	↑↑	—	—	NA	NA

ANATOMY	Body Weight		Brain Weight		Cortical Thickness		AChE	
	M	F	M	F	M	F	M	F
SEX								
Pre Stroke	↑↑	—	—	—	↑	↓	↑	↑
Post Stroke	↑↑	—	↑↑	—	↓	↓	↑	↑
Post Handle	↑	—	—	—	↓	—	↑	↑

EXPERIMENT 4: P3 LESION

BEHAVIOR	Water Maze		Reaching		Circadian Activity		Corticosterone	
	M	F	M	F	M	F	M	F
SEX								
Pre Stroke	↑↑	↑↑	↑↑	—	—	—	↓↓	↑↑
Post Stroke	↑↑	↑↑	—	—	—		↓↓	↓↓
Post Handle	—	—	↓	—			NA	NA

ANATOMY	Body Weight		Brain Weight		Cortical Thickness		AChE	
	M	F	M	F	M	F	M	F
SEX								
Pre Stroke	↑↑	↑	—	—	↑	↓	↑	↑
Post Stroke	↑↑	↑↑	—	—		—	↑	↑
Post Handle	↓	↑	↓	—	↑	↑	—	—

Figure 5.10. Summary of the treatment effects on control and lesion animals in Experiment 4. Green arrows denote a positive effect, red arrows denote a negative effect and yellow arrows denote an effect that may be either positive or negative. Double arrows denote significant effects.

5. 6. 1. Prenatal stroking improves functional recovery

P3 lesion animals that were subjected to the prenatal stroking regimen showed impressive behavioral recovery on the water task. The improvement was a result of quicker acquisition of the task and was slightly better than the improved performance seen in animals that received postnatal tactile stimulation. Such recovery likely reflects alterations in intrinsic cortical circuits. This possibility will be investigated further with analysis of dendritic arborization and spine-density in the Golgi-Cox prepared tissue.

Prenatally stroked males also showed improved reaching performance but the effect was more dramatic in the early operates. A note of caution is in order here, however. Although the male rats in this experiment showed remarkable improvement in reaching performance, there were only five males in the test group and so the results should be viewed as preliminary until this result is replicated.

5. 6. 2. Prenatal stroking and Postnatal handling have different effects on males and females

It is interesting to note the sexually dimorphic nature of prenatal stroking and postnatal handling on both behavioral and anatomical outcome. In the prenatally stroked group the male animals showed better recovery on reaching performance, lower basal corticosterone levels, and more intense AchE staining than did females. Postnatally stroked animals did not show

this sex difference in behavioral or anatomical outcome so the sex difference is not a result of the tactile stimulation alone. In the postnatally handled group the lesion males had the worst reaching scores among all the groups tested, whereas the control females had the best performance overall. This suggests that sex hormones play a major role in the prenatal organization of brain architecture and this role can be modified by experience (for a review see McEwen, 1999). Prenatal experience can thus be considered a powerful modulator of brain organization and can (under appropriate conditions) provide prophylactic treatment for perinatal brain damage (Figure 5.11).

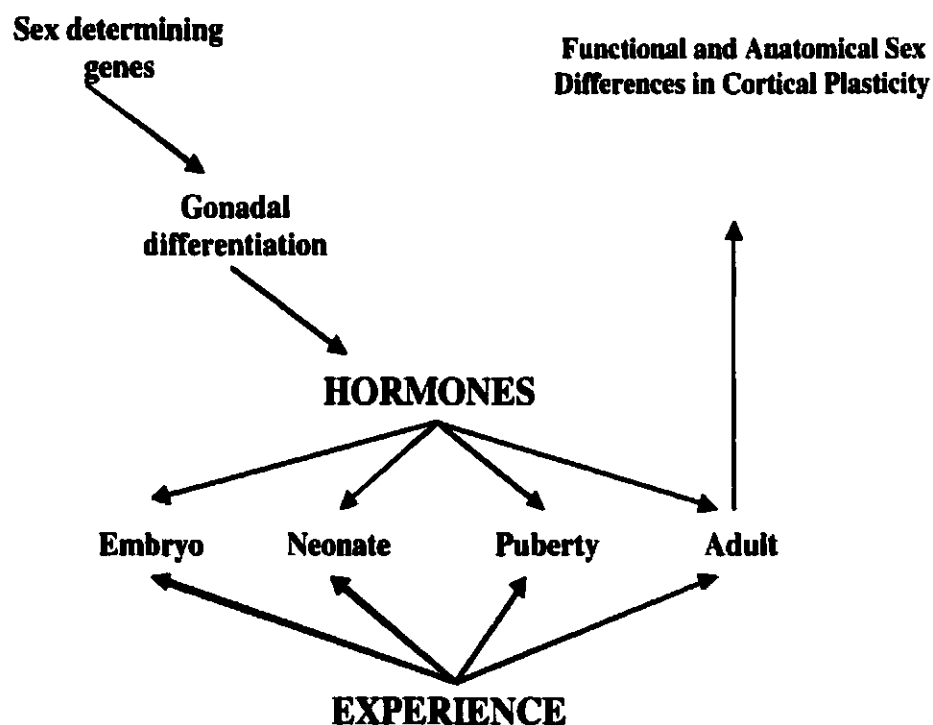


Figure 5.11. Early life experience in combination with sex hormones can have an organizational effect on brain morphology that could

ultimately affect cortical plasticity through the lifespan of the individual.

5. 6. 3. Postnatal handling does not improve functional recovery after early brain damage

Although the effects of postnatal tactile stimulation on functional outcome after early brain damage have been shown to be remedial, it was not clear what effect simple removal of the pups from the nest has on the recovery process. Work by Meaney and colleagues (1988) has shown that this intervention during the pre-weaning period has a powerful lifelong effect on corticosterone levels and ultimately on neuronal sparing in hippocampus. The reduction of hippocampal neuronal loss during senescence results in improved spatial performance for the postnatal handled rats when compared to untreated controls. As our postnatal tactile stimulation treatment involved removing the pups from the nest prior to stroking, it was clear that we needed to test postnatally handled animals to control for "removal from the nest" as a factor. The results of this experiment suggest that postnatal handling is not equivalent to the "no treatment" condition. Although postnatal handling did not facilitate recovery of lesion animals, it was not without effect. Postnatally handled animals fared worse on water maze performance than did the untreated animals, yet postnatally handled female controls had the best performance on the Whishaw reaching task of all the groups tested. Postnatal handling also caused a further decrease in brain

weight and body weight after lesion in male subjects. Many mechanisms seem to be involved in mediating the effects of postnatal handling. Meaney et al. (1991) have shown that postnatal handling causes a drop in body temperature and changes in tactile, visual, and olfactory stimulation. These changes affect the hypothalamic-pituitary-thyroid axis to induce an increase in circulating thyroxine. It is thought that thyroxine stimulates the serotonergic system, which acts on target hippocampal cells to increase glucocorticoid receptors. Increased glucocorticoid receptor level in the hippocampus is associated with enhanced negative feedback control over hypothalamic- pituitary adrenal function and ultimately results in a reduced physiological response to stress. Neonatal handling has also been found to upregulate dynorphin peptides (Ploj, Pham, Bergstrom, et al., 1999) and it is thought that this anatomical change may contribute to the attenuated fearfulness these animals exhibit in novel environments as adults. How these changes interact with early frontal cortex damage is not known but it is clear that the interaction is not beneficial. In addition, studies by Moore (1992) and Caldji, Tannenbaum, Sharma, et al. (1998) have shown that maternal stimulation has powerful effects on neural organization. Disruption of normal maternal behavior patterns could thus have an impact on resultant brain organization and behavior.

5. 6. 4. Prenatal and postnatal experience alters basal corticosterone levels

All treatment groups examined showed changes in corticosterone levels as compared to untreated animals. The postnatal treatments (handling and stroking) had similar effects on corticosterone expression. In both of these treatment groups there was a lower level of urine corticosterone than that seen in untreated animals. This result is not surprising in view of the work of Denenberg et al. (1967) and Meaney et al. (1988) that showed removal of the pups from the nest in infancy caused lifelong changes in responsiveness of the hypothalamic-pituitary-adrenal axis to stress. The result of prenatal stimulation was unexpected, however. Although the males showed a decline in corticosterone production similar to that seen in the postnatal treatment groups, the females showed an elevated response. Prenatal stimulation is very different from postnatal stimulation in many ways, but the principal difference is that the stimulation is indirect for the most part. The mother receives the direct tactile stimulation and her response to this stimulation thus modulates the nature of the experience that the pups receive. In addition, the pups do not experience the same drop in body temperature, as do the animals that are removed from the nest. Meaney et al. (1991) have shown that the drop in body temperature with postnatal handling is required to elicit the change in the corticosterone response. Thus, there must be alternate mediating factors that are responsible for the changed corticosterone expression in the prenatally stimulated animals.

5. 6. 5. Stroking and changes in Acetylcholinesterase levels

Tactile stimulation both pre- and postnatally causes increased lifelong expression of the acetylcholinesterase enzyme in the cerebral cortex. This could mean that alterations in cholinergic systems within the cortex may have occurred resulting in an upregulation of acetylcholine (ACh). Increases in cortical ACh are associated with many physiological events such as spontaneous arousal, sensory stimulation, and attention (Acquas, Wilson, & Fibiger, 1998), all of which are thought to be involved with mechanisms supporting the plasticity of learning and memory. Alternatively, increased expression of AchE may have non-cholinergic implications. AchE has been found to possess the ability to influence membrane conductance, enhance excitatory amino acid transmission, and to hydrolyze peptides (Appleyard, 1992). It is feasible that increased expression of AchE in tactilely- stimulated animals reflects increased potential for learning. Although tactile stimulation did not appear to affect the performance of control animals on any of the tasks we used in this experiment it could be that more sensitive tasks of learning are required to see slight behavioral improvements.

It appears that the expression of AchE may change with time after cortical lesion and this interacts with experience. The postnatal tactilely- stimulated animals showed a robust increase in AchE staining the first week following injury and then a decline in staining by the second post-operative week. By sampling AchE in pre- and postnatal tactilely- stimulated animals

more than three weeks postoperatively we may have missed a lesion-induced elevation of AchE. Clearly, future studies should examine AchE intensity at several time points after cortical injury and/or environmental stimulation to gain a better understanding of the nature and functional consequences of changes in the normal patterns of expression.

There is a transient expression of AchE in non- cholinergic systems during the development of the nervous system that is associated with outgrowth and extension of neurites before synaptogenesis (Appleyard, 1992). It may be the case that this early upregulation of AchE after cortical injury reflects increased outgrowth of neural processes and increased plasticity.

6. GENERAL DISCUSSION

Previous work on the nature of cortical plasticity following brain injury has offered many insights as to how, when, and to what extent functional recovery can be expected (e.g., Kolb & Gibb, 1990, Kolb & Gibb, 1991; Kolb & Wishaw, 1991; Kolb, Gibb, & van der Kooy, 1994). During the processes of development and aging, there are periods during which cortical plasticity is relatively high, and periods during which cortical plasticity is relatively low. Periods of high cortical plasticity coincide with anatomical events such as neurogenesis, gliogenesis, synaptogenesis, apoptosis and synaptic pruning. Periods of low plasticity coincide with periods during

development or aging wherein the genesis of neurons, glia and synapses is limited (Figure 1.1).

The developmental age of the animal at the time of injury has a major impact on resulting spontaneous recovery because the potential for cortical plasticity changes over the lifetime of the animal. In rats, prenatal brain injury at E18 is permissive for good behavioral recovery (despite the resultant anatomical abnormalities) whereas early postnatal injury results in a rather poor behavioral outcome. Postnatal injury in the second postnatal week allows for significant spontaneous behavioral recovery yet injuries in adulthood yield poor functional outcome (Table 1.1).

It is clear that the consequences of brain injury depend on many other factors including lesion size and location. If a cortical lesion is large enough to include an entire functional area, the resulting deficits will be more severe on tasks relevant to the injury than if some of the area is spared. Likewise, cortical lesions within functional areas should interfere with behaviors supported by that area but leave other unrelated behaviors relatively intact (e.g., Kolb, 1995).

Experience in the form of sensory stimulation, pharmacological interventions, and steroidal hormones (gonadal and glucocorticoids) also plays a fundamental role in cortical plasticity and recovery of function. The impact that these interventions have on resulting behavioral recovery seems primarily dependent on the degree of spontaneous recovery the animals experiences as a result of the injury. For example, animals that show a high

degree of spontaneous recovery (after brain injury at P7-P10) have little room for improvement and as a result derive little additional benefit (if any) from therapeutic intervention. Animals that experience very poor spontaneous recovery (after brain injury at P1-P5) have much room for improvement and thus benefit the most from experiential treatments.

The primary goal of this work was to determine how environmental interventions can be used to improve behavioral recovery after early brain damage and how the effectiveness of these interventions can be maximized. In order to achieve this goal a model of brain injury, that allows very limited spontaneous behavioral recovery, was used. Behavioral recovery was then assessed following environmental stimulation. A model of brain injury that allows good spontaneous recovery was also used, to determine if further recovery could be achieved and if there were negative consequences of environmental intervention.

6.1. Novel findings

Several important findings have resulted from these studies. First, experiential interventions are effective in stimulating cortical plasticity and functional recovery and the accompanying morphological changes are diverse in nature. Second, the age of the animal at time of treatment plays a major role in resulting plasticity and/or recovery. Third, there are sex differences in response to experiential stimulation that also seem dependent on both the age at lesion and the age at treatment. Fourth, damaged brains

sometimes respond differently than normal brains to environmental treatment. Fifth, early environmental intervention alters HPA responsivity and may ultimately have an impact on functional recovery. Sixth, some behavioral deficits are more resistant to remediation than are others.

6.1.1. Experiential treatments stimulate cortical plasticity and functional recovery

Rearing animals in a complex environment causes a variety of changes within the brain including neuronal (Greenough & Volkmar, 1973) and glial changes, alterations in vasculature (Sirevaag & Greenough, 1988), and changes in basal levels of growth factors (Torasdotter et al., 1998) and acetylcholinesterase (Rosenzweig, 1971). It is assumed that the morphological changes induced by experience underlie cortical plasticity and enable processes such as learning, memory, and functional recovery to take place.

By providing complex housing for normal and early brain-damaged animals, morphological and behavioral changes are stimulated that can improve functional recovery in lesion animals. Following complex rearing, lesion animals show an improved performance on the water task and females with P7 lesions show functional improvement on skilled reaching. Although control animals tend not to show behavioral improvements (particularly on the water task) following complex housing, this may be an inability to reliably detect minor changes in their performance with the assessments used. Complex rearing of both lesion and sham-operated

animals induces anatomical changes such as increased brain weight and altered neuronal morphology. Within pyramidal neurons there are increases in dendritic arborization and length, and altered spine density. With the exception of spine density, all of these measures showed qualitatively similar changes regardless of the age of the animal at treatment. Quantitatively, complex rearing had a larger anatomical impact on animals introduced to "enriched" housing as young adults as compared to complex rearing as juveniles or in senescence. Changes in spine density related to complex rearing are age, sex, and area- dependent. Thus, males exposed to complex housing in adulthood or senescence showed increases in spine density whereas exposure to complex housing as juveniles reduced spine density. In females, spine density was reduced in parietal neurons regardless of the age at treatment but was increased in occipital neurons when complex housing commenced in adulthood. There seems to be an additional benefit of complex housing in senescence. Aging causes dendritic atrophy and decreased spine density that is reversed by exposure to complex environments.

Postnatal tactile stimulation has a tremendous impact on the functional recovery of animals with perinatal cortical injury. This early sensory stimulation also mediates changes in the behavior of control animals. For example, tactilely- stimulated control animals show improved accuracy on skilled reaching performance as compared to non-stimulated controls. Animals with early lesions of either posterior parietal or frontal

cortex show behavioral improvements on the water task and frontals show increased accuracy on the reaching task following tactile stimulation.

(Animals with PPC removals are not impaired in reaching performance [Kolb & Cioe, 1998].) Anatomical changes also result from this early stimulation.

Although control animals showed no change in neuronal dendritic length as a result of tactile stimulation, this was not the case with the lesion animals.

Both perinatal frontal and parietal lesions cause a decrease in dendritic length but this loss of arbor was completely reversed by tactile stimulation in the PPC operates. The effect of tactile stimulation in the frontal animals was to diminish the loss of dendritic arbor associated with the lesion.

Tactile stimulation has an enigmatic effect on spine density in control animals. Following early stimulation spine density is reduced in both apical and basilar neuronal fields. Normally, reduced spine density is associated with functional loss but this is clearly not the case with the control animals. (Recall that control animals subjected to tactile stimulation showed enhanced reaching performance compared to unstimulated controls.) Although loss in spine density usually indicates synaptic loss this may not be the case for the stimulated controls. There is a possibility that the early stimulation is preventing some of the normal neuronal loss caused by apoptosis in the developing brain. In a study done by Young, Lawlor, Leone, Dragunow, and During (1999), the effect of environmental enrichment on apoptosis in the hippocampus was studied. Juvenile rats placed in complex housing showed a 45% decrease in spontaneous apoptosis in the granule cell layer of the

hippocampus after only three weeks of exposure. This supports the notion that environmental enrichment may reduce spontaneous apoptosis in neocortical areas as well. If more neurons were available to make contacts with other cells the number of spines required per cell would be reduced. Further investigation of the possibility of increased neuronal packing density is underway.

Tactile stimulation produced a different effect on spine density in lesion animals. Animals with early cortical injury show a decline in spine density that is reversed or diminished following tactile stimulation. Acetylcholinesterase levels also rise after tactile stimulation. AchE is secreted by neurons and their dendrites in an activity- dependent manner (eg., Weston & Greenfield, 1986; Appleyard, Vercher, & Greenfield, 1988) and this secreted AchE functions to reinforce synaptic efficacy and to promote growth and differentiation of postsynaptic surfaces (Rodriguez-Ithurrealde, Henley, & Bravo, 2000). Thus, it is not surprising that the most dramatic increase in AchE expression occurs in the first week post-surgery, which is also coincidental with the first week of stimulation. Increased expression of AchE may also reflect upregulation of acetylcholine expression and acetylcholine is thought to play an important role in cortical synaptic plasticity.

Acetylcholine may induce plasticity via two mechanisms (Rasmusson, 2000). First, acetylcholine may act as a facilitator of N-methyl-D-aspartate (NMDA)-mediated plasticity by altering presynaptic regulation of transmitter release or by its depolarizing action on postsynaptic neurons. Second,

acetylcholine may recruit the same second messengers that are engaged by NMDA receptor mediated plasticity such as Ca^{2+} or Ca^{2+} /calmodulin - dependent protein kinase II, independent of the NMDA receptor (for a review see Rasmusson, 2000). The elevated expression of AchE is maintained over the lifetime of the stimulated animals and may thus reflect a lifelong enhanced potential for synaptic plasticity.

Postnatal tactile stimulation also affects cortical thickness in male animals. Although males develop significantly heavier brains following postnatal tactile stimulation, they show reduced cortical thickness (an effect that is also significant). It seems puzzling that a heavier brain would possess a thinner cortex but it might be the case that the stimulated brain has larger subcortical structures such as the thalamus. Clearly, more anatomical analysis is required of these brains to allow us a better understanding of the qualitative and quantitative effects of tactile stimulation on brain morphology.

Prenatal tactile stimulation also has a potent effect on the functional recovery of animals that sustain an early brain injury. A curious finding is that male and female offspring seem to respond to this prenatal intervention differently. Whereas both male and female lesion offspring show a functional benefit of prenatal tactile stimulation on the water task, males alone show enhanced performance on skilled reaching. Control males also show reduced circadian activity following prenatal stimulation. Although prenatal stimulation has no effect on brain weight, both control and lesion

males show increased body weight as adults. Acetylcholinesterase expression also increases in animals exposed to prenatal stimulation and the expression is sex-dependent. Male animals show denser AchE staining following prenatal stimulation than do females.

Postnatal handling does not offer the same therapeutic benefit as do prenatal and postnatal tactile stimulation for animals with early cortical injury, but it is not without effect. Normal females show significantly improved accuracy on the skilled reaching task whereas lesion males had a diminished performance as compared to untreated lesion males. Water maze acquisition was somewhat impaired in the postnatally handled lesion animals and their latency scores remained higher than untreated animals in the last few days of testing. Both male and female lesion animals showed significantly reduced overall circadian activity following the postnatal handling treatment. Postnatal handling reduces basal levels of corticosterone (Denenberg et al., 1967; Meaney et al., 1991) and as a result produces animals that show superior adaptive responses to stress. It is thought that task-induced stress may interfere with performance on some tests of skilled reaching, particularly in females (C. Gonzalez, 2000, unpublished observations) and it could be that the improved performance seen in the control female group is a result of enhanced responsivity of these animals to stressful situations. It is likely that the reduced circadian activity seen by postnatally handled lesion animals is also a reflection of the altered responses to stress. Postnatal handling had very little anatomical effect on either

control or P3 lesion animals. Although there was a non-significant trend for increased cortical thickness in the lesion animals, there was also a non-significant trend for reduced brain weight in the same group. AchE expression did not differ from that seen in untreated animals and there was no evidence of divergent responses between the sexes.

6.1.2. The age of the subject at time of treatment plays a major role in resulting plasticity and/or recovery

Just as age at injury is an important factor in predicting functional outcome, so too is age at treatment. Complex housing has a more potent effect on functional recovery if the animal is exposed to this experiential treatment as a juvenile rather than in adulthood. This age-dependency may reflect a relatively higher degree of cortical plasticity in the younger animals and thus a greater potential for reorganization. Postnatal tactile stimulation is also a powerful therapeutic intervention for early brain damage. Delivery of the treatment closer to the time of insult seems to increase the curative potential of the therapy. It could be that chemokines and cytokines that are released following CNS injury interact with neurotrophins that are expressed as a result of experiential stimulation to produce enhanced cortical plasticity. Heightened plasticity might allow for greater synaptic reorganization and ultimately lead to improved functional recovery.

The finding that prenatal tactile stimulation can have a tremendous impact on the functional outcome of animals that sustain later brain damage

demonstrates that experiential treatment can act as a prophylaxis for later brain injury. It may be that the fetal animal is at the developmental stage that possesses the most potential for cortical plasticity and, under certain conditions, this potential can be expressed. This notion is supported by the finding that brain damage at E18 results in virtually normal behavior despite persistent abnormal brain morphology (Kolb, Cioe, & Muirhead, 1998). Thus, experience during fetal development may have a larger impact on plasticity and brain organization than experience in the postnatal period.

6.1.3. There are sex differences in response to experiential stimulation that may depend on age at lesion and age at treatment

The effect of gonadal hormones on the organization of the brain begins before birth and continues throughout life. According to McEwen (1999) "testosterone secretion during embryonic, neonatal, peripubertal and adult life masculinizes and defeminizes the brain. Estrogen actions in the female brain activate functions that have been allowed to develop in the absence of testosterone... experiences during the lifespan interact with the hormone actions ...". Thus, experiential treatment may have different consequences on both behavior and brain morphology of males and females.

In Experiment 1, it was shown that although complex housing increase dendritic arborization in juvenile males there is no similar increase in juvenile females. Likewise, although complex housing causes an increase in spine density in young adult males, the reverse is seen in young adult

females. Juraska and colleagues have done a number of experiments examining the differential effects of experience on male and female rats (for a review see Juraska, 1990) and she concludes that the effects are not simple. The story becomes even more complicated when brain injury is introduced as a factor. Although complex housing increases reaching performance in P7 lesion females (Experiment 2C), it has no beneficial effect on males with similar lesions yet these males show better recovery on water maze performance than do the females. Both males and females show a lesion-induced reduction in dendritic branching and although complex rearing restores some of the lost arbor, the effect of experience is larger in the males.

In Experiment 4, prenatal tactile stimulation improved the reaching performance of P3 male animals, but P3 females showed no benefit whereas handling improved the reaching performance of control females and had no effect on reaching in males. One of the most dramatic sex differences noted in this experiment was the divergent glucocorticoid response to prenatal tactile stimulation. Whereas prenatally-stimulated males showed a similar reduced response in basal glucocorticoid levels to that seen in postnatally stroked and postnatally handled animals, prenatally-stroked females showed a markedly *elevated* response. A response in this direction could have negative implications for these females as hippocampal neurons are susceptible to long term elevation of glucocorticoids (Meaney et al. 1988). Thus, during the aging process, continued glucocorticoid hypersecretion causes negative feedback insensitivity and loss of hippocampal neurons. These deficits form a self-

perpetuating cascade that causes accelerated hippocampal damage and spatial memory impairments in senescence.

6.1.4. Damaged brains respond differently than normal brains to environmental treatment

Environmental stimulation interacts with brain injury and as a result brain morphology in lesion animals is sometimes different than that observed in normal animals following treatment. This result is not surprising when one considers the potency of early lesion in defining resultant brain architecture. Although altered connectivity helps sustain functional improvement after lesion by replacing lost connections with new or altered contacts, even animals that show poor functional recovery may show extensive aberrant connectivity (Kolb et al., 1994). Experiential treatment may thus have an altered template to act upon. Differences that arise in normal and lesion brains after stimulation may be manifested in a variety of ways including spine density, dendritic length, cortical thickness, AchE expression, and brain weight. The implications of the direction of these changes may also be different in normal and brain-injured animals. Thus, further studies that detail the changes in brain morphology and their resulting functional consequences following injury and treatment are required.

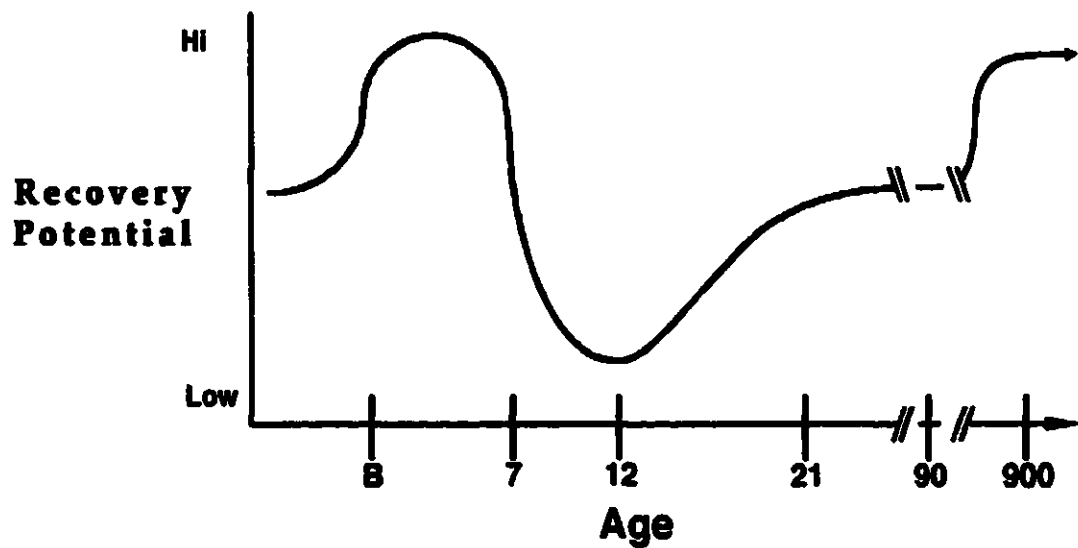


Figure 6.1. Depiction of the recovery potential of rats at particular developmental ages. When spontaneous recovery is low, recovery potential is high, as is effectiveness of experiential treatments.

6.1.5. Early environmental intervention alters HPA responsivity and may ultimately have an impact on functional recovery

Early experience affects resulting emotionality in rats. As early as 1957, work by W. Thompson showed that prenatal maternal anxiety could influence the emotionality of her offspring. Denenberg and Whimbey (1963) did a follow-up study that showed these emotional modifications were mediated through both mother-fetus interactions and postnatal mother-young interactions. Denenberg and colleagues continued to research the effect of early postnatal experience on emotionality and stress responses and found that by simply removing animals for 3 minutes per day from their nest

(handling), a dramatic effect on basal corticosterone levels and responsivity to novel stimuli was elicited (Denenberg et al., 1967). Parallel studies established that the pituitary adrenocortical response to stress was reduced in handled animals (Levine et al., 1967) and work by Meaney and colleagues showed that basal glucocorticoid levels and glucocorticoid receptor levels in hippocampus and frontal cortex were altered by the handling experience (Meaney, Aitken, van Berkel, Bhatnagar, & Sapolsky, 1988; Diorio, Viau, & Meaney, 1993).

In Experiment 4, it was shown that basal glucocorticoid levels are not altered by early frontal cortex damage (a result that is surprising in light of the discovery that frontal cortex is a major site of glucocorticoid receptor II [Diorio, Viau, & Meaney, 1993] and plays a role in the negative feedback loop of glucocorticoid production [Sullivan & Gratton, 1999]), but are responsive to both prenatal and postnatal tactile stimulation. Prenatal alteration of HPA organization might occur by the transfer of stress hormones through the placenta into the developing fetus. The prenatal tactile stimulation may serve as a low-level stressor of the pregnant dam and thus her emotional tone could influence development of her offspring. Postnatal tactile-stimulation requires removal of the pups from the maternal nest for 15-minute periods thrice daily. Similarly, handled rats are removed from the nest for similar time periods but with no subsequent additional tactile stimulation and these animals also show lowered basal glucocorticoid levels. It may be the case that lower basal glucocorticoid levels in the postnatal tactile stimulation animals is primarily a result of removal from the maternal nest

and develops in a manner similar to that seen in handled animals. Taken together, these experiments demonstrate that stress hormones play a primary role in brain organization during both the prenatal and postnatal periods of life and the resulting morphology has behavioral and anatomical implications that extend through adulthood into senescence.

6.1.6. Some behavioral deficits that result from early brain damage are more resistant to remediation than are others.

The degree of recovery observed following therapeutic intervention is task-dependent. Whereas cognitive tasks such as spatial navigation seem to benefit dramatically from environmental stimulation, other tasks such as skilled reaching are more resistant to improvement. This is likely due to the differing nature of the systems underlying the behavior. Cortico-cortical connections are largely responsible for spatial performance and are more easily reorganized than are the cortico-spinal connections that primarily support motor ability. Although behavioral recovery is sometimes seen on skilled reaching it is possible that spared motor cortex is subserving the observed restoration of function. Even when there is evidence of restored motor capacities it may be that the animal is using compensatory strategies rather than exhibiting specific recovery of the affected behavior. The reaching task employed to assess recovery of skilled limb use relies on an endpoint measure of success. In instances where skilled forelimb use is improved by environmental treatments it is impossible to say the lesion animals

“recovered” skilled forelimb use as the tactics they rely on to successfully retrieve food in this task may be entirely different from those used by normal animals. Perhaps a more detailed analysis of limb use after brain injury and subsequent treatment would reveal which aspects of motor behavior are benefited by therapy.

6.2. Proposed mechanisms of experiential treatment as therapy for early brain damage

Experience can be a powerful modulator of brain organization and can (under appropriate conditions) provide an effective treatment for perinatal brain damage. The following proposals detail the mechanisms that may underlie the observed changes in functional recovery and brain morphology after complex housing or pre- or postnatal tactile stimulation.

6.2.1. Mechanisms that may mediate the *complex housing* treatment effect

1. *Alterations in AchE expression.* Rosenzweig and colleagues have done a number of studies on the effects of postweaning complex rearing on normal animals and have observed a consistent upregulation of AchE expression in the brains of animals exposed to “enriched” environments (Rosenzweig, 1971). AchE has been implicated in alterations of synaptic efficacy, and growth and differentiation of neurons (Appleyard, 1993; Bravo et al., 2000). Increased expression of AchE may also mimic increased

acetylcholine expression and acetylcholine is thought to play an important role in mediating cortical plasticity (Rasmusson, 2000).

2. *Alterations in growth factor expression.* Work by Torasdotter and colleagues (1998) demonstrated that mRNA for nerve growth factor (NGF) is upregulated following environmental enrichment. Recent work by Carro, Nunez, Busiguina, and Torres-Aleman (2000) showed that exercise induces increased uptake of serum insulin-like growth factor (IGF-1) by specific groups of neurons and these neurons show increased spontaneous firing and prolonged sensitivity to afferent stimulation. Rats living in condominiums show increased levels of activity that could induce similar increased neuronal uptake of serum IGF-1. Altered expression of growth factors like these may be partly responsible for increased cortical plasticity and subsequent functional improvements in early brain-damaged animals that have been exposed to complex housing.

3. *Stimulation of neurogenesis.* Enriched housing can stimulate neurogenesis in the dentate gyrus of adult mice (Kempermann, Kuhn, & Gage, 1997). It may be that neocortical neurogenesis is also stimulated by enriched housing, particularly in brain-injured animals.

4. *Decreased apoptosis.* Environmental enrichment has been shown to inhibit spontaneous apoptosis in the hippocampus (Young et al., 1999) and although a similar effect has not been demonstrated in the neocortex, it is not unreasonable to propose that it may exist.

5. *Increased neural activity.* Neural activity is required in the visual system for pattern formation and synaptic plasticity (Katz & Shatz, 1996) and it is thought that NMDA receptors mediate this developmental cortical plasticity (Catalano, Chang, & Shatz, 1997). Exposure to complex housing increases an animal's exposure to a variety of sensory stimuli that should increase neuronal activity in cortical areas related to processing sensory stimulation. It may be the case that increased neuronal activity resulting from complex housing may alter the developmental regulation of NMDA receptors resulting in increased cortical plasticity. A study of the effects of early experience on cortical dendrites (Schapiro & Vucovich, 1970) showed that exposure to noise, flashing light, tactile stimulation (mechanical and thermal) and electric shock resulted in increased spine density on dendrites of neurons located in visual and auditory cortex.

6. *Altered stress reactivity.* Mohammed, Henriksson, Soderstrom et al. (1993) demonstrated that enriched housing caused changes in expression of glucocorticoid receptors in hippocampus similar to that seen after neonatal "handling" of rats. Upregulation of these receptors increases the sensitivity of the negative feedback loop in the stress response and causes lowered basal levels of glucocorticoid expression. This altered response in HPA reactivity could have a beneficial effect on resulting functional recovery.

6.2.2. Mechanisms that may mediate the *prenatal* tactile stimulation treatment effect

1. *Increased availability of basic Fibroblast Growth Factor (bFGF).* The role of bFGF in nervous system development and differentiation has been established (Raballo, Rhee, Lyn-Cook, et al., 2000) but the developmental significance and trophic role of bFGF is not limited to the nervous system. Many other organ systems rely on bFGF for proliferation and differentiation and among these is the skin. Dermal fibroblasts synthesize bFGF and following a wound expression of bFGF is increased. It may be the case that tactile stimulation also upregulates the expression of bFGF in the skin and increased availability of bFGF results in higher serum levels of bFGF. A recent study by Wagner, Black and DiCicco-Bloom (1999) demonstrated that systemic levels of bFGF regulated neurogenesis in the brains of newborn and adult rats by crossing the blood brain barrier to mediate its effects. If this were the case, one might suppose that additional bFGF may become available to fetal brain tissue by transfer through the placenta.

2. *Maternal expression of low levels of glucocorticoids.* Tactile stimulation of a rat may mediate an increased expression of glucocorticoid in response to the novelty of human handling and stroking. In recent studies by Hansson, Cintra, Belluardo, et al. (2000) and Mochetti, Spiga, Hayes, Isackson, & Colangelo (1996), glucocorticoids were found to regulate the gene expression of bFGF, Brain derived neurotrophic factor (BDNF), NGF, and neurotrophic factor- 3 (NT-3) in rat dorsal hippocampus and neocortex.

Glucocorticoids may (via placental transfer) become available to developing fetal brain where they could play a role in resulting expression of genes for neurotrophic factors.

3. *Increased neural activity.* (See section 6.2.1 for discussion.)

4. *Alteration in maternal care and mother-pup interactions.* A study done by Vilecas, Bell, Wright, and Kufner (1976) on the effect of “handling” on maternal behavior following the return of the pups to the nest, noted that “ultrasonic signaling by the infants and careful assessment of the mother’s hormonal and behavioral state during the separation from her pups is necessary to gain a better interpretation of maternal responsiveness and how it may influence later differences in pup outcome measures”. More recent work by Liu, Diorio, Day, Francis, & Meaney (2000) showed that mothers that engage in a higher frequency of licking and grooming of their pups, and an arched back nursing posture, reared offspring that showed enhanced performance on tests of spatial learning and memory and showed a correlated increased expression of NMDA receptors and mRNA for BDNF. Clearly, maternal behavior has a major impact on resultant offspring behavior and brain morphology and should be considered as a possible factor in stimulating functional recovery from early injury.

5. *Increased expression of acetylcholinesterase.* Prenatal tactile stimulation causes increased expression of AchE in the brains of offspring (see section 6.2.1. for discussion of these possibilities), and males show higher

levels of expression of this enzyme than do females. This may indicate that males derive more benefit from prenatal tactile stimulation than do females.

6.2.3. Mechanisms that may mediate the *postnatal* tactile stimulation treatment effect

1. *Increased serum lactate.* Serum lactate is reported to be a preferred metabolic substrate for neonatal rat brain tissue (Dombrowski, Swiatek, & Chao, 1989) and tactile stimulation during the first week of life has been shown to increase circulating levels of serum lactate (Aslami, Pickens, & Hoath, 1997). Reorganization of the brain is likely very energy demanding and tactile stimulation provides a way of increasing metabolic substrate availability.

2. *Increased tissue ornithine decarboxylase.* Ornithine decarboxylase (ODC) is an enzyme that is involved in the control of nervous system development. Each area within the brain has a characteristic pattern of expression during ontogenesis and areas with high levels of ODC expression are undergoing rapid growth (Slotkin & Bartolome, 1986). Maternal deprivation is associated with decreased levels of ODC (Wang, Bartolome, & Schanberg, 1996) whereas tactile stimulation induces increased expression of this enzyme (Evoniuk, Kuhn, & Schanberg, 1979). Thus, tactile stimulation may influence the growth and development of brain through a mechanism related to ODC activity.

3. *Increased acetylcholinesterase.* Tactile stimulation induces upregulation of AchE and may increase cortical plasticity through mechanisms related to AchE or acetylcholine. (See section 6.2.1 for a discussion.)

4. *Stimulation induced increased availability of bFGF.* (See section 6.2.2 for discussion.)

5. *Increased neural activity.* (See section 6.2.1 for discussion.)

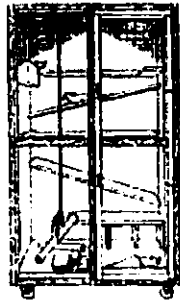
6. *Transmission of maternal glucocorticoids through nursing.* Levine and co-workers have determined that maternal stress during nursing can lead to altered neuroendocrine maturation and behavior in her pups (Levine, 1967) presumably through transmission of stress hormones in milk. (See section 6.2.2 for a discussion of the effects of glucocorticoids on neurotrophic factor gene expression.)

7. *Alteration in maternal care and mother-pup interactions.* (See section 6.2.2 for a discussion.)

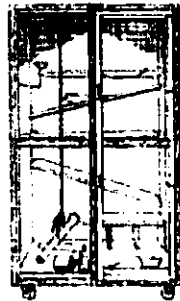
6.3. Conclusion

Experiential treatments can provide effective therapy for early brain damage. Complex housing and postnatal tactile stimulation provide excellent restorative therapy after early brain injury by effecting a host of anatomical changes within the central nervous system (Figure 6.2). Recently, a paper by Rampon, Jiang, Dong, et al. (2001) detailed the effect of environmental enrichment on the genome. Using the oligonucleotide

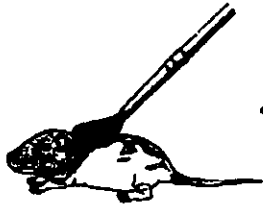
microarray technique to analyze gene expression in the brain, the authors determined that no less than 100 genes were affected by complex housing. The time course of expression levels of the genes varied over the period of exposure to complex housing with initial increased expression of genes coding proteins involved in macromolecule synthesis and processing and enzymes involved in DNA, RNA and protein processing. Genes coding for proteins involved in apoptosis were downregulated whereas genes coding for proteins involved in the formation of new synapses and reorganization or strengthening of existing synapses were upregulated. Late changes in the genome affected transcripts for proteins involved in neuronal transmission and structural changes. These findings support the notion that enriched experience produces many diverse effects on the subsequent behavioral repertoire and brain morphology of animals exposed to it. Remarkably, prenatal stimulation works extremely well as a *prophylaxis* for later perinatal brain injury. Although the mechanisms underlying this treatment effect are not known, it is very likely that the induced changes are also numerous and diverse. Thus, experiential treatment provides both prophylactic and curative therapy that allows significant improvement in functional performance of animals that have sustained early brain injury.



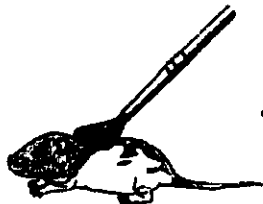
+ P4 Frontal Lesion = - Behavioral Recovery
- Dendrite and Spine Growth



+ P7 Frontal Lesion = - No Effect



+ P4 Frontal/ Parietal Lesion = - High Behavioral Recovery
- High Dendrite and Spine Growth



+ P3 Frontal Lesion = - High Behavioral Recovery
- Increased AchE



+ P3 Frontal Lesion = - High Behavioral Recovery
- Increased AchE

Figure 6.2. Summary of the effectiveness of experiential treatment in stimulating behavioral recovery.

6.4. Future Directions

As the benefit of environmental therapies in promoting behavioral recovery from early brain damage have been clearly established, it is appropriate to investigate, in detail, the anatomical mechanisms that support the improved behavioral outcome. Tissue from animals that have undergone pre- and postnatal tactile stimulation and postnatal handling (and appropriate controls) has been prepared for a Golgi analysis of potential morphological changes in neurons. Tissue has also been prepared to allow immunohistochemical analysis of potential changes in astrocytes (glial fibrillary acid protein), bFGF expression, and distribution of glucocorticoid receptors.

As serotonin is implicated in the restructuring of glucocorticoid receptor expression in the first week of life (Meaney, Mitchell, Aitken, et al., 1991), administration of a serotonergic agonist to early brain damaged animals may be an effective means of promoting behavioral recovery in adulthood. Following behavioral testing, urine analysis to determine basal corticosterone levels and preparation of brain tissue for immunohistochemical determination of glucocorticoid receptor expression would be undertaken.

Additional proposed experiments include placing pregnant dams in a "complex" housing condition, then testing the offspring on various tasks as adults. Tissue processing would proceed as in earlier experiments so a comparison can be made of the relative effectiveness of these various

interventions. The potential of prenatal complex housing as a form of therapy for early brain injury would also be explored. If functional recovery is supported by this treatment, a detailed analysis of brain tissue from the early operates would follow to determine what biochemical and morphological changes have occurred.

The significant functional improvement seen in early- lesion animals born to tactilely- stimulated mothers raises the question of whether or not administration of exogenous pharmacological compounds might also influence functional recovery after perinatal brain injury. External administration of neurotrophic factors such as bFGF and IGF-1 may promote behavioral recovery by inducing cortical plasticity. If tactile stimulation increases systemic bFGF, exogenous administration of bFGF should result in similar functional improvement and anatomical changes as seen with tactile stimulation.

Other pharmacological compounds that may promote behavioral recovery in animals with early brain injury are psychomotor stimulants. Animals that sustain brain injury as adults show improved functional performance after exposure to moderate doses of nicotine (Brown, Gonzalez, & Kolb, 2000). Prenatal administration of nicotine may also influence behavioral recovery in developing rats.

The possibility that these prenatal and postnatal treatments may stimulate neurogenesis in brain-damaged animals will be investigated by using bromodeoxyuridine (a mitotic marker) to label newly- formed cells and

then using appropriate antibodies to determine whether the new cells are neurons or glia. Similarly, environmental treatment may alter the course of apoptosis during development. Thus, by using antibodies to proteins involved in apoptotic processes we could determine how these processes are influenced by experiential therapy.

In a preliminary experiment designed to look at the relative effectiveness of prelesion versus postlesion tactile stimulation in animals with P10 frontal cortex lesions, we noted that prelesion stimulation seemed to have negative effects on resulting functional performance. If there are circumstances that contraindicate therapeutic intervention, it is important to know what these circumstances are. Further studies employing an animal model that allows good spontaneous recovery (i.e., frontal cortex damage at P7-P10 in rats) should provide the means to elucidate when environmental and pharmacological interventions are not appropriate.

In sum, my proposal is to continue to investigate the biochemical and anatomical changes that underlie the behavioral recovery seen in animals that have been exposed to early environmental stimulation to gain a better understanding of the mechanisms that allow this recovery to occur. If an insight can be gained into how the brain makes use of the environmental stimulation it receives to initiate plasticity in systems that normally fail to show any plasticity following damage, we could propose alternate therapies that work on the same substrates to produce similar or even more dramatic recovery.

References:

Appleyard, M. E. (1992). Secreted acetylcholinesterase: non-classical aspects of a classical enzyme. *Trends in Neuroscience*, 15, 485-490.

Appleyard, M.E., Vercher, J. L. & Greenfield, S. A. (1988). Release of acetylcholinesterase from the guinea-pig cerebellum in vivo. *Neuroscience*, 25, 133-138.

Aslami, M. M., Pickens, W.L., Hoath, S.B. (1997). Effect of tactile stimulation on serum lactate in the newborn rat. *Pediatric Research*, 41, 857-861.

Bailey, C. H & Kandel, E. R. (1993). Structural changes accompanying memory storage. *Annual Review of Physiology*, 55, 397-426.

Black, J. E., Greenough, W. T., Anderson, B. J., & Isaacs, K.R. (1987). Environment and the aging brain. *Canadian Journal of Psychology*, 41, 111-130.

Bock, J. & Braun, K. (1998). Differential emotional experience leads to pruning of dendritic spines in the forebrain of domestic chicks. *Neural Plasticity*, 6, 17-27.

Bolles, R.C., & Woods, P.J. (1964). The ontogeny of behavior in the albino rat. *Animal Behavior*, 12, 427-441.

Brain Injury Association of Alaska. (2000). *Alaska Statistics: The basics of brain injury*. Web Site. Available:
<http://www.alaska.net/~drussell/biaak/akstats1.html>

Buell, S. J., & Coleman, P. D. (1985). Regulation of dendritic extent in developing and aging brain. In C. Cotman (Ed.). *Synaptic plasticity*, pp.311-333. New York, NY: Raven.

Bravo, S. O., Henley, J. M., & Rodriguez-Ithurralde, D. (2000).

Acetylcholinesterase potentiation of AMPA receptors: a possible route to synaptic plasticity. *6th Internet World Congress for Biomedical Sciences*, Web Site. Available:<http://www.uclm.es/inabis2000/symposia/files/045/session.htm#intro>

Bland, B. H. & Cooper, R. M. (1969). Posterior neocortical ablation in the rat: age at operation and experience. *Journal of Comparative Physiology and Psychology*, 69, 345-354.

Brown, R. W., Gonzalez, C.L.R., & Kolb, B. (2000). Nicotine improves Morris water task performance in rats given medial frontal cortex lesions. *Pharmacology, Biochemistry and Behavior*, 67, 473-478.

Caldji, C., Tannenbaum, B., Sharma, S., Francis, D. D., Plotsky, P M., & Meaney, M. J. (1998) Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. *Proceedings of the National Academy of Science, USA*, 95, 5335-5340.

Canadian Institute of Child Health. (2000). *The health of Canada's Children: A CICH profile. Low birth weight*. Web Site. Available:<http://www.cich.ca/lowbirth.htm>.

Carro, E., Nunez, A., Busiguina, S., & Torres-Aleman, I. (2000). Circulating insulin-like growth factor I mediates effects of exercise on the brain. *Journal of Neuroscience*, 20, 2926-2933.

Catalano, S.M., Chang, C.K., & Shatz, C.J. (1997). Activity-dependent regulation of NMDAR1 immunoreactivity in the developing visual cortex. *Journal of Neuroscience*, 17, 8376-8390.

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

Dean, K. (2000). Protocol for radioimmunoassay of corticosterone in urine. Unpublished.

DeFilipe, J. & Jones, E.G. (1988). *Cajal on the cerebral cortex: An annotated translation of the complete writings*. New York, NY: Oxford University Press.

Denenberg, V.H. & Whimbey, A.E. (1963). Behavior of adult rats is modified by the experiences their mothers had as infants. *Science*, 142, 1192-1193.

Denenberg, V. H., Brumaghim, J. T., Haltmeyer, G. C., & Zarrow, M. X. (1967). Increased adrenocortical activity in the neonatal rat following handling. *Endocrinology*, 81, 1047-1052.

Diamond, M., Lindner, B., & Raymond, A. (1967). Extensive cortical depth measurements and neurons size increases in the cortex of environmentally enriched rats. *Journal of Comparative Neurology*, 131, 261-268.

Diorio, D. Viau, V. & Meaney, M.J. (1993). The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. *Journal of Neuroscience*, 13, 3839-3847.

Dombrowski, G. J., Swiatek, K R., & Chao, K.L. (1989). Lactate, 3-hydroxybutyrate, and glucose as substrates for the early postnatal rat brain. *Neurochemical Research*, 14, 667-675.

Evoniuk, G.E., Kuhn, C.M., & Schanberg, S.M. (1979). The effect of tactile stimulation on serum growth hormone and tissue ornithine decarboxylase activity during maternal deprivation in rat pups. *Communications in Psychopharmacology*, 3, 363-370.

Field, T. M., Schanberg, S. M., Scafildi, F. A., Bauer, C. R., Vega-Lahr, N., Garcia, R., Nystrom, J., & Kuhn, C. M (1986). Tactile/kinesthetic stimulation effects on preterm neonates. *Pediatrics*, 77, 654-658.

Finger, S. & Almlı, C.R. (1984). Early brain damage. Vol. 2. *Neurobiology and behavior*. New York, N.Y.: Academic Press.

Finger, S. & Almlı, C. R. (1988). Margaret Kennard and her "principle" in historical perspective. In S. Finger, T.E. Le Vere, C.R. Almlı, & D.G. Stein (Eds.), *Brain injury and recovery: Theoretical and controversial issues* (pp.

117-132). New York, N.Y.: Plenum Press.

Flood D. G. (1993). Critical issues in the analysis of dendritic extent in aging humans, primates, and rodents. *Neurobiology of Aging*, 14, 649-654.

Gibb, R. & Kolb, B. (1998). A method for Golgi-Cox staining of Vibratome cut tissue. *Journal of Neuroscience Methods*, 79, 1-4.

Globus, A., Rosenzweig, M. R., Bennett, E. L., Diamond, M. C. (1973). Effects of differential experience on dendritic spine counts in rat cerebral cortex. *Journal of Comparative Physiological Psychology*, 82, 175-181.

Greenough, W. T., Black, J. E., & Wallace, C. (1987). Experience and brain development. *Child Development*, 58, 539-559.

Greenough, W. T., & Chang, F.-L. F. (1989). Plasticity of synapse structure and pattern in the cerebral cortex. In A. Peters & E. Jones (Eds.) *Cerebral Cortex: (Vol 7)*, pp. 391-440. New York, NY: Plenum Press.

Greenough, W. T. & Volkmar, F. R. (1973). Pattern of dendritic branching in occipital cortex of rats reared in complex environments. *Experimental Neurology*, 40, 491-504.

Gould, E., McEwen, B. S., Tanapat, P., Galea, L. A. M., and Fuchs, E. (1997). Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. *Journal of Neuroscience*, 17, 2492-2498.

Hansson, A.C., Cintra, A., Belluardo, N., Sommer, W., Bhatnagar, M., Bader, M., Ganten, D., & Fuxe, K. (2000). Gluco- and mineralcorticoid receptor-mediated regulation of neurotrophic factor gene expression in the dorsal hippocampus and the neocortex of the rat. *European Journal of Neuroscience*, 12, 2918-2934.

Hebb, D.O. (1947). The effects of early experience on problem solving at maturity. *American Psychologist*, 2, 737-745.

Hebb, D. O. (1949). *The organization of behavior*. New York, NY: McGraw-Hill.

John F. Kennedy Center for Research on Human Development, Vanderbilt University. (1999). *Brain Plasticity*. Web Site. Available: <http://www.vanderbilt.edu/kennedy/topics/brainpl.html>

Johnston, M. V. (1988). Biochemistry of Neurotransmitters in Cortical Development. In A. Peters & E. G. Jones (Eds.), *Development and maturation of cerebral cortex*. New York, NY: Plenum Press.

Juraska, J. M. (1984). Sex differences in dendritic responses to differential experience in the rat visual cortex. *Brain Research*, 95, 27-34.

Juraska, J. M. (1986). Sex differences in developmental plasticity of behavior and the brain. In W. T. Greenough, & J. M. Juraska (Eds.) *Developmental neuropsychobiology*, pp. 409-422. New York NY: Academic Press.

Juraska, J. M. (1990). The structure of the cerebral cortex: Effects of gender and the environment. In B. Kolb & R. Tees (Eds.) *The cerebral cortex of the rat*, pp. 483-506. Cambridge, MA: MIT Press.

Juraska, J. M., Fitch, J. M., Henderson, C., & Rivers, N. (1985). Sex differences in the dendritic branching of dentate granule cells following differential experience. *Brain Research*, 333, 73-80.

Juraska, J. M., Fitch, J. M., & Washburne, D. L. (1989). The dendritic morphology of pyramidal neurons in the rat hippocampal CA3 area. II. Effects of gender and experience. *Brain Research*, 479, 115-121.

Juraska, J. M. & Meyer, M. (1986). Behavioral interactions of postweaning male and female rats with a complex environment. *Developmental Psychobiology*, 19, 493-500.

Katz, L. C. & Shatz, C. J. (1996). Synaptic activity and the construction of cortical circuits. *Science*, 274, 1133-1138.

Kempermann, G., Kuhn, H.G. & Gage, F.H. (1997). More hippocampal neurons in adult mice living in an enriched environment. *Nature*, 386, 493-495.

Kennard, M. (1940). Relation of age to motor impairment in man and subhuman primates. *Archives of Neurology and Psychiatry*, 44, 77-397.

Kiyono, S. Seo, M.L., Shibagaki, M., & Inouye, M. (1985). Facilitative effects of maternal environmental enrichment on maze learning in rat offspring. *Physiology & Behavior*, 43, 431-435.

Kolb, B. (1987). Recovery from early cortical damage in rats. I. Different behavioral and anatomical effects of frontal lesions at different ages of neural maturation. *Behavioral Brain Research*, 25, 205-220.

Kolb, B. (1990). Sprouting and recovery of function. In B. Kolb & R. Tees (Eds.) *The cerebral cortex of the rat*. Cambridge, MA: MIT Press.

Kolb, B. (1995). *Brain Plasticity and Behavior*. Mahwah, NJ: Lawrence Erlbaum Associates.

Kolb, B. (1999). Towards an ecology of cortical organization: experience and the changing brain. *Building a bridge from the laboratory to the clinic*. Berlin: Springer.

Kolb, B. & Cioe, J. (1998). Absence of recovery or dendritic reorganization after neonatal posterior parietal lesions. *Psychobiology*, 26, 134-142.

Kolb, B. & Cioe, J. (2000). Recovery from early cortical damage in rats. VIII. Earlier may be worse: behavioral dysfunction and abnormal cerebral morphogenesis following perinatal frontal cortical lesions in the rat. *Neuropharmacology*, 39, 756-764.

Kolb, B., Cioe, J., & Muirhead, D. (1998). Cerebral morphology and functional sparing after prenatal frontal cortex lesions in rats. *Behavioral Brain Research*, 91, 143-155.

Kolb, B. & Elliott, W. (1987). Recovery from early cortical damage in rats. II. Effects of experience on anatomy and behavior following frontal lesions at 1 or 5 days of age. *Behavioral Brain Research*, 26, 47-56.

Kolb, B. & Gibb, R. (1990). Anatomical correlates of behavioral change after neonatal prefrontal lesions in rats. *Progress in Brain Research*, 85, 241-256.

Kolb, B. & Gibb, R. (1991). Environmental enrichment and cortical injury: Behavioral and anatomical consequences of frontal cortex lesions in rats. *Cerebral Cortex*, 1, 189-198.

Kolb, B. & Gibb, R. (1993). Possible anatomical basis of recovery of spatial learning after neonatal prefrontal lesions in rats. *Behavioral Neuroscience*, 107, 799-811.

Kolb, B., Gibb, R., & Gorny, G. (2001). Experience-dependent changes in dendritic spine arbor and spine density in neocortex vary with age and sex. Submitted to *Neurobiology of Learning and Memory*, in press.

Kolb, B., Gibb, R., Gorny, G., & Ouellette, A. (1996). Experience dependent changes in cortical morphology are age dependent. *Society for Neuroscience Abstract*, 22, 1133.

Kolb, B., Gibb, R., & van der Kooy, D., (1994). Neonatal frontal cortical lesions in rats alter cortical structure and connectivity. *Brain Research*, 645, 85-97.

Kolb, B., Gibb, R., Gorny, G., & Whishaw, I. Q. (1998). Possible brain regrowth after cortical lesions in rats. *Behavioral Brain Research*, 91, 127-141.

Kolb, B., Gorny, G., & Gibb, R. (1994). Tactile stimulation enhances recovery and dendritic growth in rats with neonatal frontal lesions. *Society for Neuroscience Abstract*, 20, 1430.

Kolb, B. & McClimans J. (1986). A process for cryostat sectioning of Golgi-Cox tissue. *Stain Technology*, 61, 379-380.

Kolb, B., & Nonneman, A. J. (1976). Functional development of the prefrontal cortex continues into adolescence. *Science*, 193, 335-336.

Kolb, B., & Nonneman, A. J. (1978). Sparing of function in rats with early prefrontal cortex lesions. *Brain Research*, 151, 135-148.

Kolb, B., Petrie, B., & Cioe, J. (1996). Recovery from early cortical damage in rats. VII. Comparison of the behavioural and anatomical effects of medial prefrontal lesions at different ages of neural maturation. *Behavioral Brain Research*, 79, 1-13.

Kolb, B. & Stewart, J. (1991). Sex-related differences in dendritic branching of cells in the prefrontal cortex of rats. *Journal of Neuroendocrinology*, 3, 95-99.

Kolb, B. & Stewart, J. (1995). Changes in neonatal gonadal hormonal environment prevent behavioral sparing and alter cortical morphogenesis after early frontal cortex lesions in male and female rats. *Behavioral Neuroscience*, 109, 285-294.

Kolb, B., Stewart, J., & Sutherland, R. J. (1997). Recovery of function is associated with increased spine density in cortical pyramidal cells after frontal lesions or noradrenaline depletion in neonatal rats. *Behavioral Brain Research*, 89, 61-70.

Kolb, B., Sutherland, R. J., & Wishaw, I. Q. (1983c). Neonatal hemidecortication or frontal cortex ablation produce similar behavioral sparing but opposite effects upon morphogenesis of remaining cortex. *Behavioral Neuroscience*, 97, 154-158.

Kolb, B., & Wishaw, I. Q. (1981). Neonatal frontal lesions in the rat: sparing of learned but not species-typical behavior in the presence of reduced brain weight and cortical thickness. *Journal of Comparative and Physiological Psychology*, 95, 863-879.

Kolb, B. & Whishaw, I. Q. (1991) Mechanisms underlying behavioral sparing alter neonatal electroencephalographic activity. *Brain Dysfunction*, 4, 75-92.

Kolb, B. & Whishaw, I. Q. (1998). Brain plasticity and behavior. *Annual Review of Psychology*, 49, 43-64.

Kolb, B. & Whishaw, I. Q. (2000). *Introduction to brain and behavior*. New York, NY: Worth Publishers.

Konorski, J. (1948) *Conditioned reflexes and neuron organization*. Cambridge: Cambridge University Press.

Levine, S. (1967). Maternal and environmental influences on the adrenocortical response to stress in weanling rats. *Science*, 156, 258-260.

Levine, S., Haltmeyer, G., Karas, G., and Denenberg, V. (1967). Physiological and behavioral effects of infantile stimulation. *Physiology and Behavior*, 2, 55-59.

Liu, D., Diorio, J., Day, J. C., Francis, D. D., & Meaney, M. J. (2000). Maternal care, hippocampal synaptogenesis and cognitive development in rats. *Nature Neuroscience*, 3, 799-806.

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D. D., Freedman, A., Sharma, S., Pearson, D., Plotsky, P. M., and Meaney, M. J. (1997). Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science*, 239, 1659-1662.

Meaney, M. J., Aitken, D. H., and Sapolsky, R. M. (1987). Thyroid hormones influence the development of hippocampal glucocorticoid receptors in the rat: a mechanism for the effects of postnatal handling on the development of the adrenocortical stress response. *Neuroendocrinology*, 47, 278-283.

Meaney, M. J., Aitken, D. H., van Berkel, C., Bhatnagar, S., and Sapolsky, R. M. (1988). Effect of neonatal handling on age-related impairments associated with the hippocampus. *Science*, 239, 766-768.

Meaney, M. J., Mitchell, J. B., Aitken, D. H., Bhatnagar, S., Bodnoff, S. R., Iny, L. J., & Sarrieau, A. (1991). The effects of neonatal handling on the development of the adrenocortical response to stress: implications for neuropathology and cognitive deficits in later life. *Psychoneuroendocrinology*, *16*, 85-103.

McEwen, B. S. (1999). Permanence of brain sex differences and structural plasticity of the adult brain. *Proceedings of the National Academy of Science, USA*, *96*, 7128-7130.

Miklyaeva, E. I., Kolb, B., & Whishaw, I. Q. (1997). Effects of unilateral striatal dopamine-depletion and behavioural experience on the dendritic arborization of neurons in rat sensorimotor cortex. *Society for Neuroscience Abstracts*, *23*, 93.4.

Mohammed, A.H., Henriksson, B.G., Soderstrom, S., Ebendal, T., Olsson, T., & Seckl, J. R. (1993). Environmental influences on the central nervous system and their implications for the aging rat. *Behavioral Brain Research*, *57*, 183-191.

Mocchetti, I., Spiga, G., Hayes, V.Y., Isackson, P.J., & Colangelo, A. (1996). Glucocorticoids differentially increase nerve growth factor and basic fibroblast growth factor expression in the rat brain. *Journal of Neuroscience*, *16*, 2141-2148.

Moore, C. (1992). The role of maternal stimulation in the development of sexual behavior and its neural basis. *Annals of the New York Academy of Science*, *662*, 160-177.

Morris, R. G.M. (1981). Spatial localization does not require the presence of local cues. *Learning and Motivation*, *12*, 239-260.

Morris, R. G.M. (1984). Developments of a water-maze for studying spatial learning memory in the rat. *Journal of Neuroscience Methods*, *11*, 47-60.

- Nicoll, A. & Blakemore, C. (1993). Patterns of local connectivity in the neocortex. *Neural Computation*, 5, 665-680.
- Patel, S. N., Rose, S. S. R., & Stewart, M. G. (1988). Training induced dendritic spine density changes are specifically related to memory formation processes in the chick, *Gallus domesticus*. *Brain Research*, 463, 168-173.
- Paxinos, G. & Watson, D. (1982). *The rat brain in stereotaxic coordinates*. New York, N.Y.: Academic Press.
- Pfaff, D. W. (1965). Morphological changes in the brains of adult male rats after neonatal castration. *Journal of Endocrinology*, 36, 415-416.
- Ploj, K., Pham, T. M., Bergstrom, L., Mohammed, A. H., Henriksson, B. G., & Nylander, I. (1999). Neonatal handling in rats induces long-term effects on dynorphin peptides. *Neuropeptides*, 33, 468-474.
- Raballo, R., Rhee, J., Lyn-Cook, R., Leckman, J.F., Schwartz, M.L. & Vaccarino, F.M. (2000). Basic fibroblast growth factor (Fgf2) is necessary for cell proliferation and neurogenesis in the developing cerebral cortex. *Journal of Neuroscience*, 20, 5012-5023.
- Ramon y Cajal, S. (1894). The fine structure of the nerve centers. *Proceedings of the Royal Society of London*, 55, 444-468.
- Ramon y Cajal, S. (1928). *Degeneration and regeneration of the nervous system*. London: Oxford University Press.
- Rampon, C., Jiang, C.H., Dong, H., Tang, Y., Lockhart, D. J., Schultz, P.G., Tsien, J. Z., & Hu, Y. (2001). Effects of environmental enrichment on gene expression in the brain. *Proceedings of the National Academy of Science*, 97, 12880-12884.
- Rasmusson, D.D. (2000). The role of acetylcholine in cortical synaptic plasticity. *Behavioral Brain Research*, 115, 205-218.

Rodriguez-Itharrude, D., Henley, J.M., & Bravo, S.O. (2000). Mutual regulation between glutamate receptors and acetylcholinesterase. *6th Internet World Congress for Biomedical Sciences*. Web site. Available: <http://www.uclm.es/inabis2000/symposia/files/051/index.htm>

Rosenzweig, M. R. (1971). Effect of environment on development of brain and behavior. In E. Tobach, L. Aronson & E. Shaw (Eds.), *The biopsychology of development*. pp. 303-342. New York, NY: Academic Press.

Rosenzweig, M. R. & Bennett, E. L. (1978). Experiential influences on brain anatomy and brain chemistry in rodents. In G. Gottlieb (Ed.) *Studies on the development of behavior and the nervous system*. pp.289-387. New York, NY: Academic Press.

Rosenzweig, M. R., Krech, D., Bennett, E. L., & Diamond, M. C. (1962). Effects of environmental complexity and training on brain chemistry and anatomy: A replication and extension. *Journal of Comparative Physiological Psychology*, 55, 429-37.

Schanberg, S., & Field, T. (1987). Sensory deprivation stress and supplemental stimulation in the rat pup and preterm human neonate. *Child Development*, 58, 1431-1447.

Schapiro, S. & Vukovich, K. R. (1970). Early experience effects upon cortical dendrites: a proposed model for development. *Science*, 167, 292-294.

Schulkin, J. Ed. (1989). *Preoperative events: Their effects on behavior following brain damage*. Hillsdale, NJ: Erlbaum.

Sholl, D. A. (1956). *The organization of the cerebral cortex*. London: Methuen.

Sirevaag, A. M. & Greenough, W. T. (1988). A multivariate statistical summary of synaptic plasticity measures in rats exposed to complex, social and individual environments. *Brain Research*, 441, 386-392.

Slotkin, T.A. & Bartolome, J. (1986). Role of ornithine decarboxylase and the polyamines in nervous system development: A review. *Brain Research Bulletin*, 17, 307-320.

Stewart, J. & Kolb, B. (1988). The effects of neonatal gonadectomy and prenatal stress on cortical thickness and asymmetry in rats. *Behavioral Neural Biology*, 49, 344-360.

Stewart, J. & Kolb, B. (1994). Dendritic branching in cortical pyramidal cells in response to ovariectomy in adult female rats: Suppression by neonatal exposure to testosterone. *Brain Research*, 654, 149-154.

Stiles, J. (2000). Spatial cognitive development following prenatal or perinatal focal brain injury. In H.S. Levin & J. Grafman (Eds.), *Cerebral Reorganization of function after brain damage*. Oxford: Oxford University Press.

Sullivan, R.M. & Gratton, A. (1999). Lateralized effects of medial prefrontal cortex lesions on neuroendocrine and autonomic stress responses in rats. *Journal of Neuroscience*, 19, 2834-2840.

Sutherland, R. J., Whishaw, I. Q. & Kolb, B. (1983). A behavioral analysis of spatial localization following electrolytic, kainate-, or colchicine-induced damage to the hippocampal formation in the rat. *Behavioral Brain Research*, 7, 133-153.

Thompson, W. R. (1957). Influence of prenatal maternal anxiety on emotionality in young rats. *Science*, 125, 698-699.

Uylings, H. B. M., Van Eden, C. G., Parnavelas, J. G., & Kalsbeek, A. (1990). The prenatal and postnatal development of the rat cerebral cortex. In B.Kolb & R. Tees (Eds.), *The cerebral cortex of the rat*. Cambridge, MA: MIT Press.

Vargha-Khadem, F., O'Gorman, A.M., & Watters, G.V. (1985). Aphasia and handedness in relation to hemispheric side, age at injury, and severity of cerebral lesion during childhood. *Brain*, 108, 77-696.

Villablanca, J. R., Hovda, D. A., Jackson, G. F., & Infante, C. (1993). Neurological and behavioral effects of unilateral frontal cortical lesion in fetal kittens: II. Visual system tests, and proposing "an optimal developmental period" for lesion effects. *Behavioral Brain Research*, *57*, 79-92.

Villegas, R., Bell, R. W., Wright, L. & Kufner, M. (1976). Effect of handling on maternal behavior following return of pups to the nest. *Developmental Psychobiology*, *10*, 323-329.

Wagner, J. P., Black, I. R., & DiCicco-Bloom, E. (1999). Stimulation of neonatal and adult brain neurogenesis by subcutaneous injection of basic fibroblast growth factor. *Journal of Neuroscience*, *19*, 6006-6016.

Walsh, R. (1982). *Towards and ecology of the brain*. New York, NY: SP Medical books.

Wallhauser, E. & Scheich, H. (1987) Auditory imprinting leads to differential 2-deoxyglucose uptake and dendritic spine loss in the chick rostral forebrain. *Developmental Brain Research*, *31*, 29-44.

Wang, S., Bartolome, J. V., & Schanberg, S.M. (1996). Neonatal deprivation of maternal touch may suppress ornithine decarboxylase via downregulation of the proto-oncogenes *c-myc* and *max*. *Journal of Neuroscience*, *16*, 836-842.

Weston, J. & Greenfield, S. A. (1986). Release of acetylcholinesterase in the rat nigrostriatal pathway: Relation to receptor activation and firing rate. *Neuroscience*, *17*, 1079-1088.

Whishaw, I. Q., Dringenberg, H. C., & Pellis, S. M. (1990). Spontaneous forelimb grasping in free feeding by rats: Motor cortex aids limb and digit positioning. *Behavioral Brain Research*, *48*, 113-125.

Whishaw, I. Q., Pellis, S. M., Gorny, B. P., & Pellis, V. C. (1991). The impairments in reaching and the movements of compensation in rats with motor cortex lesions: An endpoint, videorecording, and movement notation analysis. *Behavioral Brain Research*, *42*, 77-91.

Whishaw, I. Q., O'Connor, W. T., Dunnett, S. B. (1986). The contributions of motor cortex, nigrostriatal dopamine and caudate-putamen to skilled forelimb use in the rat. *Brain*, 109, 805-843.

Will, B., & Kelche, C. (1992), Environmental approaches to recovery of function from brain damage: A review of animal studies (1981 to 1991). In F. Rose & D. Johnson (Eds.) *Recovery from brain damage: reflections and directions*. New York, NY: Plenum Press.

Yanai, J. (1979). Delayed maturation of the male cerebral cortex in rats. *Acta Anatomica*, 104, 335-339.

Young, D., Lawlor, P. A., Leone, P., Dragunow, M., & During, M. J. (1999). Environmental enrichment inhibits spontaneous apoptosis, prevents seizures and is neuroprotective. *Nature Medicine*, 5, 448-453.

Zilles, K. (1985). *The cerebral cortex of the rat*. Berlin: Springer.