

BASIC FIBROBLAST GROWTH FACTOR IN THE INJURED BRAIN

SHARON R. ROWNTREE
(Bachelor of Science, University of Lethbridge, 1994)

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

MASTER OF SCIENCE

UNIVERSITY OF LETHBRIDGE
LETHBRIDGE, ALBERTA
November, 1995

© Sharon R. Rowntree, 1995

DEDICATION

To Lori

....who inspired the questions....

ABSTRACT

Basic fibroblast growth factor (bFGF) has been implicated in the brain's trophic response to injury. This thesis examined the effects of endogenous bFGF on brain plasticity and recovery of behavioral function following cortical injury in adult rats. The first experiment investigated the post-lesion time course of the astrocytic expression of bFGF. Subsequent experiments examined the effects of injury-induced bFGF on neuronal morphology, cortical morphology, and post-lesion behavioral deficits.

Following motor cortex injury, endogenous bFGF prevented neuritic degeneration in layer V pyramidal neurons in Zilles' area Fr2 and promoted recovery of function in the Whishaw Reaching Task. Housing rats in an enriched environment prior to cortical injury enhanced the expression of bFGF but did not increase cortical thickness nor reduce post-lesion behavioral deficits (relative to laboratory-housed rats). Collectively, these experiments indicate that injury-induced bFGF plays a role in potentiating recovery from brain damage. This implies that bFGF may be beneficial as a treatment following brain injury.

ACKNOWLEDGEMENTS

Nothing happens just as one fears or hopes.

THEODOR HERZL.

This thesis could not have been completed without the encouragement and support of the two main men in my life - Bryan and Bryon. I am grateful to my supervisor, Bryan Kolb, for providing me with the guidance, opportunities, and facilities that enabled me to conduct my research and to write my thesis. I appreciate the patience and understanding shown by my husband, Bryon Rowntree, who quietly assumed many responsibilities so that I could "research and write".

The successful completion of my graduate studies is due to the efforts of many people and I wish to thank all of them: Ian Wishaw and Jim Thomas for being supportive members of my supervisory committee; Robbin Gibb for keeping me on track (physically and psychologically); Margaret Forgie for the "psychological ice-packs"; Grazyna Gorny, Bogden Gorny, Alan Oullette, and Karen Cazal for technical advice; and Evelyn Field, Brenda Coles, and Lori Smith for being there.

I acknowledge with thanks the financial assistance of the Natural Sciences and Engineering Research Council of Canada who provided a post-graduate scholarship and the University of Lethbridge Council on Graduate Studies who appointed me a Graduate Assistant during the first year of my studies.

TABLE OF CONTENTS

Title Page.....	i
Signature Page.....	ii
Dedication.....	iii
Abstract.....	iv
Acknowledgements.....	v
Table of Contents.....	vi
List of Tables.....	vii
List of Figures.....	viii
General Introduction.....	1
Research Approach.....	28
Experiment 1.....	31
Experiment 2.....	39
Experiment 3.....	64
Experiment 4.....	74
General Discussion.....	101
References.....	107
Appendix.....	123

LIST OF TABLES

Table	Description	Page
1.	Compensatory mechanisms that may follow brain damage. (After Kolb and Whishaw, 1990)	5
2.	Summary of brain weights of animals used in Experiment 4.	92
3.	Summary of body weights of animals used in Experiment 4.	93

LIST OF FIGURES

Figure	Description	Page
1.	A typical neuron, showing some of its major physical features. (From Kolb and Whishaw, 1990)	4
2.	Time course of events following CNS injury. (After Nieto-Sampedro and Cotman, 1985)	24
3.	Drawings of coronal sections in representative brains of adult rats with unilateral motor cortex lesions, showing astrocytic distribution of bFGF.	36
4.	Mean number of bFGF-reactive and GFAP-reactive astrocytes in an area medial to unilateral motor cortex lesions.	37
5.	Representation of the right hemisphere of the rat brain at 1.7 mm posterior to bregma. (After Zilles, 1985)	46
6.	Weekly performance of anti-bFGF-treated, treatment-control, and control rats in the Whishaw Reaching Task following unilateral motor cortex lesions in the hemisphere contralateral to the preferred reaching paw.	48
7.	Mean number of bFGF-reactive astrocytes in an area medial to unilateral motor cortex lesions in the brains of anti-bFGF-treated and untreated rats.	51
8.	Mean number of GFAP-reactive astrocytes in an area medial to unilateral motor cortex lesions in the brains of anti-bFGF-treated and untreated rats.	52
9.	Mean number of total basilar dendritic branches on layer V pyramidal neurons in Zilles' area Fr2 in the brains of anti-bFGF-treated, treatment-control, and control rats.	54

10.	Mean basilar dendritic branching frequency on layer V pyramidal neurons in Zilles' area Fr2 in the brains of anti-bFGF-treated, treatment-control, and control rats.	55
11.	Mean number of spines on a 10 μ m section of basilar dendrite on layer V pyramidal neurons in Zilles' area Fr2 in the brains of anti-bFGF-treated, treatment-control, and control rats.	57
12.	Drawings of the basilar dendritic arbor of representative layer V pyramidal neurons in Zilles' area Fr2 in the brains of anti-bFGF-treated, treatment-control, and control rats.	59
13.	Drawings of basilar dendritic spine density on representative layer V pyramidal neurons in Zilles' area Fr2 in the brains of anti-bFGF-treated, treatment-control, and control rats.	60
14.	Mean number of bFGF-reactive astrocytes in an area medial to unilateral motor cortex lesions in the brains of laboratory-housed and enriched-housed rats.	69
15.	Mean number of GFAP-reactive astrocytes in an area medial to unilateral motor cortex lesions in the brains of laboratory-housed and enriched-housed rats.	71
16.	Mean number of successful reaches made by laboratory-housed and enriched-housed rats following bilateral frontal cortex lesions.	82
17.	Mean number of total reaches made by laboratory-housed and enriched-housed rats following bilateral frontal cortex lesions.	84
18.	Mean escape latency of laboratory-housed and enriched-housed rats in the place navigation task following bilateral frontal cortex lesions.	86
19.	Total escape latency of laboratory-housed and enriched-housed rats in the place navigation task following bilateral frontal cortex lesions.	87

20.	Mean escape latency of laboratory-housed and enriched-housed rats in the landmark navigation task following bilateral frontal cortex lesions.	89
21.	Total escape latency of laboratory-housed and enriched-housed rats in the landmark navigation task following bilateral frontal cortex lesions.	90
22.	Effects of enrichment on cortical thickness in the brains of laboratory-housed and enriched-housed rats following bilateral frontal cortex lesions.	94

GENERAL INTRODUCTION

One of the most significant issues being addressed during the "Decade of the Brain" (1990 - 2000) is the question of how to repair the injured brain. Brain injuries are a leading cause of death in North America and brain damage is one of the most common and severe causes of social, economic, and psychological disability.

Since the early 1970s there has been an alarming increase in the number of brain-damaged people in Canadian society. This "epidemic" can be attributed to two primary causes. First, the development of new lifesaving medical techniques has contributed to a marked increase in the number of people who sustain and survive brain injuries. Prior to the 1970s, infants with severe pre- or perinatal brain damage probably would have died, but today they survive with devastating developmental disabilities such as mental retardation, cerebral palsy, and epilepsy. Children and adults who suffer brain damage as a result of vehicle accidents, sporting injuries, strokes, or drug abuse survive the trauma but live with "permanent behavioral abnormalities that may include disorders of movement, perception, or memory; loss of language; and the alteration of social behavior and personality" (Kolb, 1989).

Second, not only are more people surviving developmental and traumatic brain damage, more individuals are reaching an age at which neurodegenerative diseases become common. The dramatic increase in life expectancy during this century, primarily through the cure of infectious diseases, has resulted in more people living with aging-related diseases such as Parkinson's disease and Alzheimer's disease.

Dr. Murray Goldstein (1990), the Director of the National Institute of Neurological Disorders and Stroke (USA), recently stated that:

Mortality from traumatic brain injury over the past twelve years has exceeded the cumulative number of American battle deaths in all wars since the founding of our country. The enormity of the problem, often referred to as the silent epidemic, becomes even clearer when we realize that the total number of head injuries is conservatively estimated at over 2 million each year. The overall economic cost to society approaches \$25 billion each year. (p. 327)

Treatment and maintenance of people with brain damage present enormous costs to society in the form of health care, social services, and special education. Unfortunately, provision of these resources diminishes neither the incidence nor the prevalence of brain injuries. Preventing brain injuries and facilitating recovery from brain damage are the obvious ways of dealing with the increasing number of people with damaged brains. For example, brain injuries in children and adults can be prevented to a large extent by education and by the use of safety devices such as vehicle seat belts and protective helmets, but complete recovery seldom occurs once the brain has been damaged.

An understanding of recovery from brain damage logically must be based on knowledge of how the brain attempts to repair itself; that is, how the brain compensates for brain damage. Mental, physical, and emotional disabilities are behavioral manifestations of the damaged brain and are due to a loss of functions that may or may not be recovered. Kolb and Whishaw (1989) defined recovery of function as "the return of behavioral functions that were once present and then lost" (p. 237). Recovery of behavioral function is thought to be correlated with a process of anatomical changes in the brain. This process of change is known as brain plasticity.

Brain plasticity is based on specific physiological and morphological mechanisms that produce changes in brain cells (neurons). Parts of neurons, especially dendrites, dendritic spines, and axons, are constantly being modified in a continuous process in which new synapses are formed and others are lost (Figure 1). It was originally believed that this plastic process was restricted to the period of early brain development but it has become apparent that dendritic and axonal plasticity are maintained throughout life.

When the brain is damaged by trauma, disease, or aging, it is thought to compensate for neuron loss by utilizing the same mechanisms of organization, growth, and guidance that are found during development, and by making changes that are essential for learning and memory. In order to understand the compensatory mechanisms underlying recovery of function (Table 1), it is important to realize that anatomical and behavioral relationships are constrained by three factors; age, environment, and reactive change.

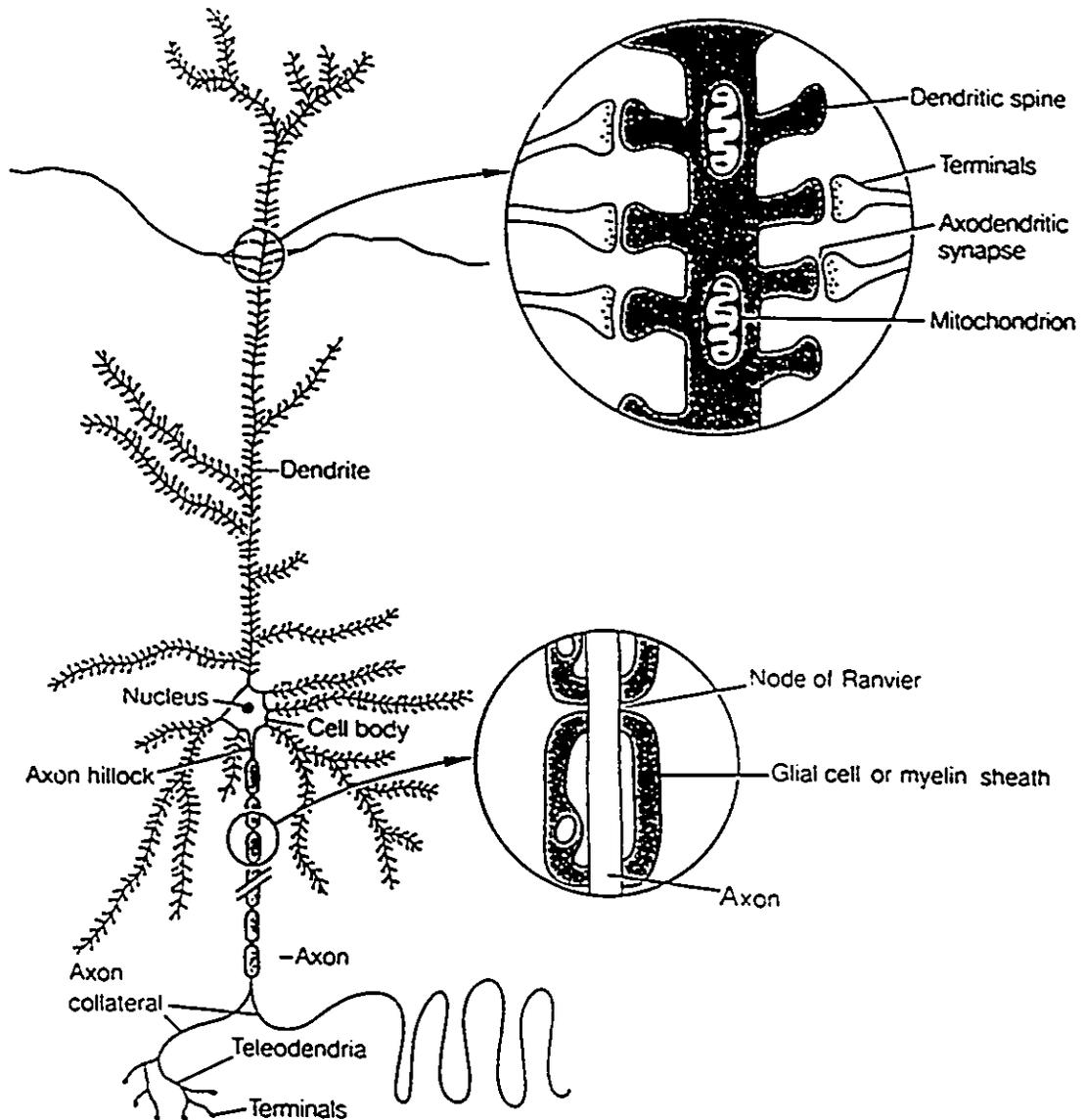


Figure 1. A typical neuron, showing some of its major physical features. (From Kolb and Wishaw, 1990)

Table 1. Compensatory mechanisms that may follow brain damage.
(After Kolb and Wishaw, 1990)

Term	Definition
Behavioral compensation	Use of a new or different behavioral strategy to compensate for a behavior lost due to brain damage, e.g., notetaking to compensate for loss of memory.
Collateral sprouting	The growth of collaterals of axons to replace lost axons or to innervate targets that have lost other afferents.
Denervation supersensitivity	The proliferation of receptors on a nerve or muscle when innervation is interrupted that results in an increase in response when residual afferents are stimulated or when chemical agonists are applied.
Disinhibition	Removal of inhibitory actions of a system usually by destroying it or blocking its action pharmacologically.
Nerve growth factor	A protein that may be secreted by glial cells that promotes growth in damaged neurons and facilitates regeneration and reinnervation by cut axons.
Regeneration	Process by which damaged neurons, axons, or terminals regrow and establish their previous connections.
Rerouting	Process by which axons or their collaterals seek out new targets when their normal destination has been removed.
Silent synapse	A hypothetical synapse that is thought to be present but whose function is not behaviorally evident until the function of some other part of the system is disrupted.
Sparing	A concept that refers to a process that allows certain behaviors or aspects of behavior to survive brain damage.
Sprouting	Growth of nerve fibers to innervate new targets, particularly if they have been vacated by other terminals.
Substitution	The idea that an unoccupied or underused area of the brain will assume functions of a damaged area (not in vogue today).
Transient collaterals	Collaterals that at some time during development innervated targets that they subsequently abandon as development proceeds.
Vicariation	A version of substitution theory that suggests that the functions of damaged areas can be assumed by adjacent areas.

The Age Factor

The age of an animal that has sustained a brain injury has a significant effect on how the animal responds to brain damage. Age when the injury is incurred is an important factor but there are two additional age-related elements that must be taken into consideration. First, behavior measured immediately following brain injury will be different than behavior after a period of recovery. Thus, testing at different ages subsequent to the injury will provide a changing picture of recovery. Second, age at behavioral assessment must be taken into account when attempting to evaluate the consequences of the brain injury.

Age at Injury

In the 1930s, Kennard (1936, 1938) reported that removal of the motor cortex in infant monkeys resulted in less behavioral impairment than similar injury in adulthood. This claim led to what has become known as the “Kennard Principle”, which states that recovery from brain damage early in life will be more complete than recovery from damage later in life.

The Kennard Principle is supported by studies of language in children. For example, Lennenberg (1967) reviewed the effects of early cortical damage on language and concluded that left hemisphere damage in the first year or two of life allows substantial recovery of language processes, presumably because there is a shift of language to the right hemisphere. In contrast, similar injuries in adults have devastating effects on various language functions with little improvement over time.

In studies of language acquisition, it appears that the earlier brain

damage is sustained, the less severe the behavioral loss, however, the Kennard Principle is only partly supported by studies of other cognitive functions. For example, Woods (1980) found that the IQs of children with brain damage in the first year of life were well below average as well as being lower than those of children who suffered brain damage later in life. Thus, the general conclusion could be that *under some circumstances* there is an advantage to having brain damage at an early age rather than later in life.

In studies of rats with bilateral injury to the motor, prefrontal, parietal, visual, or temporal cortex, Kolb and Whishaw (1989) observed that cortical injury in the first five days of life was associated with more severe behavioral loss than was similar injury at seven to ten days of life. Cortical injury at 25 days of age was again associated with severe behavioral loss, which was nearly equivalent to that seen in adults (Kolb & Whishaw, 1981a). These results indicate that there may be certain times when the brain is especially able to compensate for injury.

Recovery Period

Both humans and animals with brain damage typically demonstrate some behavioral recovery: They are most impaired immediately after the injury but the impairments gradually decline over time. And yet, recovery is seldom, if ever, complete. For example, people with anterior left hemisphere strokes may initially be unable to speak but will gradually regain some of their language functions even though they will generally demonstrate lasting impairments in finding the right words, expressing themselves clearly, or understanding complex speech by others (Eidelberg & Stein, 1974).

It is now generally agreed that brain damage early in adult life is less debilitating than when the same type of injury occurs in old age. For example, Stein and Firl (1976) found that 620 day old rats failed to exhibit the recovery of function typical of younger animals (i.e.: 180 day old rats) when the frontal cortex was removed in sequential operations. In human studies, Teuber (1975) compared the deficits of Korean War veterans on tests given one week after brain injury and those given 20 years later. Recovery from brain injury in the 17 - 20 year old group was greater than for the 21 - 25 year old group, which in turn was greater than for the group 26 years of age and older. These studies are significant for they imply that the young brain and the aging brain react differently to injury.

Experimental laboratory animals exhibit a slow improvement in the performance of cognitive tasks after cortical injury. Kolb and Gibb (1993) found that rats given medial frontal lesions on the day of birth (P1) or at 10 days of age (P10) were equally impaired at spatial localization in the Morris water task (Morris, 1980) when they were tested as soon as they were able to perform the task (P19 and P22). When the animals were examined between P56 and P59 however, the P10-lesioned animals exhibited functional recovery, whereas the P1-lesioned animals still showed deficits. The recovery of the P10-lesioned animals was correlated with an increase in the density of dendritic spines on parietal layer II-III pyramidal cells. On P22, when their performance was impaired, they showed no significant differences in dendritic measures relative to controls; on P60, when functional recovery had occurred, they showed significant increases in dendritic spine density. Thus, Kolb and Gibb (1993) demonstrated that recovery of behavioral function may be associated with

plastic changes in the brain.

Recovery of function after brain injury is thought to be correlated with multiple cellular and molecular responses. Some of these responses may be beneficial to neuronal survival and regeneration whereas others may be detrimental. For example, injury-induced glial scar formation may prevent regenerating axons from creating functional synapses. Nieto-Sampedro and Cotman (1985) argued that the key to enhancing neuronal survival and promoting neural regeneration may be found in the strict temporal order of anatomical and physiological events observed during development. This hypothesis has significant implications for treatment of brain damage. Interventions that are likely to prove successful in enhancing recovery of behavioral function following brain injury are those that are able to replicate the sequence of events that occurs in the brain during development.

Age at Assessment

Although humans and other animals typically recover some function following brain damage, behavior is never as securely established as it would be if the brain were intact. Behavior may appear to be normal under most conditions, but it is likely to deteriorate in old age.

For example, many children contracted poliomyelitis, an acute viral disease characterized by involvement of the nervous system and possible paralysis, during an epidemic in the 1950s. The victims recovered from the disease but now that they are entering their fifth decade, neurological deficits are beginning to re-appear. The brain was able to compensate for the damage caused by poliomyelitis in early life but is now unable to cope with the neuronal

loss that normally occurs with aging (Glassman & Smith, 1988).

Rats that have compensated for brain injury deteriorate more in old age than do normal rats, probably because they are unable to cope with the aging-related loss of neurons. For example, when rats that have recovered from lateral hypothalamic damage reach two years of age, they begin to lose their recovered feeding and drinking behaviors and their responsiveness to sensory stimuli. Eventually, the rats return to a condition approximating their behavior just after the lesion (Schallert, 1983). Thus, brain damage early in life may appear to be insignificant but normal aging processes may exacerbate the effects and cause devastating behavioral deficits long after functional recovery has occurred.

Summary - Age Factor

Behavioral deficits that are caused by brain injury early in life are different than those that result from damage to the mature brain. In many cases, the consequences of early injury are as debilitating (or more so) as the effects of damage later in life. In both young and mature animals, injury-induced behavioral deficits decline over time but the deficits may re-appear as the animal ages.

The Environment Factor

The idea that environmental experience might influence recovery of function following brain injury is not new. In fact, rehabilitation therapy for people with brain damage is based on the assumption that environmental stimulation may promote recovery from brain damage. The brain is affected by two types of environmental stimulation. Both external environment (factors outside the body) and internal environment (factors within the body) may stimulate plastic changes in the brain and enhance functional recovery.

External Environment

Environmental factors play an important role in the development and plasticity of brain structure and function. Since 1965, environmentally-induced modifications of brain and behavior have been documented in intact laboratory animals. Environmental "enrichment" of laboratory animals induces increased cortical weight (Diamond, Rosenzweig, Bennett, Lindner, & Lyon, 1972), increased cortical thickness (Diamond, 1967; Diamond et al., 1972), more higher order dendritic branches in neurons in occipital (Greenough & Volkmar, 1973) and frontotemporal cortex (Greenough, Volkmar, & Juraska, 1973), more glial cells (Diamond, Ingham, Johnson, Bennett, & Rosenzweig, 1976), enhanced protein synthesis in the brain (Rosenzweig, Bennett, & Diamond, 1972), more synapses per neuron (Turner & Greenough, 1985), larger synapses (West & Greenough, 1972), more dendritic spines (Globus, Rosenzweig, Bennett, & Diamond, 1973), and superior performance in complex mazes as compared with controls (Greenough, 1976).

Post-lesion Environmental Experience. Environmental enrichment appears to have dramatic effects on the brains of normal animals. Studies on the effects of such stimulation on animals with brain lesions show that environmental enrichment also affects the damaged brain.

Recovery from frontal lesions in rats varies with age (Kolb, 1987; Kolb & Elliott, 1987), lesion size (Kolb & Whishaw, 1981a, 1985), and behavioral measure (Kolb & Whishaw, 1981b, 1983). The effects of post-lesion environmental enrichment on behavior and brain morphology are also age, lesion, and task-specific. Kolb and Elliott (1987) found age-specific effects when rats were raised in an enriched environment following frontal decortication. Rats operated on at P5 developed thicker cortices in the enriched environment than rats operated on at P1. Similarly, the behavioral effects of enriched housing on the P5 operates were much more pronounced than on the P1 operates. Age-specific effects were also found in a study in which rats received hemidecortications at P1 or as adults (Whishaw, Zaborowski, & Kolb, 1984). "Beneficial" post-lesion effects were observed in the performance of the Morris water maze task, but only in the rats that were operated on as adults and not in P1 operates.

Lesion-specific effects have been demonstrated in a number of studies (Dalrymple-Alford & Kelche, 1985, 1987; Kelche, Dalrymple-Alford, & Will, 1987; Will, Kelche, & Deluzarche, 1981) in which postoperative enrichment is effective only after certain kinds of brain damage, such as lesions of the hippocampus, but not after other types of lesions, such as lesions of the afferent-efferent systems of the hippocampus.

Kolb and Gibb (1991a) demonstrated task-specific effects in rats that

sustained unilateral or bilateral frontal cortex ablations. The lesions induced several behavioral deficits that were not all attenuated or compensated for by 90 days of postoperative environmental enrichment. Anatomical analyses of the brains showed that enrichment increased brain weight and dendritic branching similarly in normal and brain-injured rats.

Will (1981) stated that "there exists...strong evidence that a postoperative or post-traumatic enriched experience significantly aids functional recovery after various kinds of brain injuries" (p. 181), but it is possible that pre-injury experience may be as important as post-injury experience in coping with brain damage.

Pre-lesion Environmental Experience. A number of studies show that the relationship between preoperative experience and postoperative test performance is specific. Singh (1973, 1974) examined the effects of specific prior experience upon food intake in rats subjected to ventromedial hypothalamic (VMH) lesions. VMH lesions normally cause excessive eating and drinking and subsequent weight gain but eating and drinking behaviors in either VMH rats trained to bar press for food or in VMH rats habituated to quinine-adulterated water preoperatively were almost identical to the behavior of controls within a few days after the lesion. In other studies (Cooper, Blochert, Gillespie, & Miller, 1972; Miller & Cooper, 1974), rats trained preoperatively on a brightness discrimination task did not require the usual extensive retraining following visual cortex lesions. These studies suggest that preoperative experience that closely approximates the postoperative test can enhance performance.

Perhaps the best evidence for a potentially nonspecific relationship

between preoperative experience and postoperative recovery comes from studies in which rats have been housed preoperatively in complex environments. In two studies (Donovick, Burrigh, & Swidler, 1973; Donovan, Burrigh, & Bensten, 1975), rats were reared in complex group cages or in isolation prior to ablation of much of the septal region (a nucleus in the limbic system that provides input to the hippocampus). Postoperative drinking, taste reactivity, and exploratory behavior were almost equivalent in operated and nonoperated complex-reared animals implying that there is an environmental effect independent of the lesion effect.

Hughes (1965) reared rats in complex group environments, group cages, or isolation cages prior to hippocampal, neocortical, or sham lesions. Complex rearing attenuated the effects of antero-dorsal hippocampal lesions on maze learning and appeared to diminish deficits produced by postero-ventral hippocampal destruction. Neocortically-damaged rats reared in complex and group environments did not differ from sham-operates in maze learning ability whereas neocortically-damaged rats reared in isolation committed nearly 50% more errors than did sham-operated isolates. In contrast to the findings of Donovan et al., (1973, 1975), Hughes' (1965) studies suggest an interaction between preoperative experience and postoperative behavioral recovery.

Taken together, these results suggest that generalized preoperative experience may have generalized postoperative effects. If this is the case, then one could predict that appropriate pre-lesion experience may protect against injury-induced functional loss or even promote recovery from brain damage.

Internal Environment

There are various trophic factors that underlie brain plasticity. Some of these factors are transported to the brain from other parts of the body and some are produced by the brain itself.

Gonadal Hormones. The central nervous system (CNS) is modified in essentially irreversible ways by gonadal hormones (testicular androgens and ovarian estradiol/progestins) secreted early in development (e.g. MacLusky & Naftolin, 1981). Studies of laboratory animals provide many examples of the influence of early exposure to sex hormones on sex differences in brain morphology (Diamond, Dowling, & Johnson, 1981; Stewart & Kolb, 1988) and subsequent adult behavior (Nordeen & Yahr, 1982). This is especially so in the cortex. Diamond et al (1981) found that the right cortex was thicker than the left in male but not in female rats. Geschwind and Galaburda (1987) proposed that testosterone increases the functional potency of the right hemisphere, whereas Stewart and Kolb (1988) argued that testosterone appears to suppress left cortex growth.

A similar pattern is present in human fetuses. For example, de Lacoste and Horvath (1985) found that as early as 13 weeks of gestational age, the entire right hemisphere is larger than the left in the male fetus, whereas in females the left prerolandic cortex is larger and more developed than the right.

It appears that the two hemispheres may not be equally asymmetric in males and females and this in turn may play a role in the effects of unilateral brain damage. Studies of stroke patients with left hemisphere damage revealed that the incidence of aphasia (lack of language abilities) is higher in men than in women (McGlone, 1980). Kimura (1983) showed that the pattern of

cerebral organization *within* each hemisphere may differ between the sexes. Although males and females are nearly as likely to be aphasic after left hemisphere damage, speech disorders occur most often in women when the anterior cortex is injured and in men when the posterior cortex is damaged. Similarly, limb apraxia (inability to make purposive movements) is associated with anterior damage to the left hemisphere in women and with posterior damage in men.

If males and females differ in their response to brain injury then it is quite possible that they also employ different mechanisms to repair brain damage. On a cellular level, Juraska, Fitch, Henderson, and Rivers (1985) found that male and female rats differed in their response to a differential environment. Female rats exhibited enhanced dendritic response in dentate gyrus cells whereas male rats developed more extensive dendritic arbor in visual cortex. This type of dendritic response could also be utilized as a mechanism of recovery from brain damage which would lead one to believe that males and females differ in the extent of their recovery of function after brain damage.

Endogenous Cortical Noradrenaline. The possibility that cortical noradrenaline (NA) might promote plasticity in the developing neocortex was first demonstrated by Kasamatsu and Pettigrew (1976). They suggested that depletion of NA in the postnatal kitten visual cortex by intraventricular infusion of the neurotoxin 6-hydroxydopamine (6-OHDA) could drastically retard plasticity (measured as a change in cortical connections in the visual system [ocular dominance shift] following occlusion of one eye [monocular deprivation]). In a later study Kasamatsu and Pettigrew (1979) demonstrated that continuous microperfusion of NA restored plasticity to the NA-depleted visual cortex. More

recently, Shirokawa and Kasamatsu (1986) showed that direct infusion of *B*-adrenergic antagonists into kitten visual cortex also suppressed ocular dominance shift after monocular deprivation.

Using laboratory rats, Kolb and Sutherland (1986) suggested that NA may play some role in plasticity after early lesions. Depletion of NA with 6-OHDA lesions on P1 failed to produce a decrease in cortical thickness, but depletion of NA on P1, P2, or P3 combined with a frontal lesion on P4 or P7 produced a significantly thinner cortex. Further, depletion of NA had no effect on cortical thickness if the cortical lesion was at P10 suggesting that NA may be beneficial only during certain periods of cortical development (Kolb & Sutherland, 1986).

Kolb and Sutherland (1992) confirmed that neonatal cortical NA depletion blocks the behavioral sparing normally observed in rats with frontal lesions at P7. This blockade of sparing was associated with a smaller brain and thinner cortex, as well as a greater reduction in dendritic branching than was observed in sham operates. In addition, NA depletion affected the spatial learning performance of only P7 rats; it did not affect normal animals, P4 frontal rats, or animals with frontal lesions in adulthood suggesting that there is a critical period during cortical development when NA is essential.

As well as being important during development, NA appears to play a role in brain plasticity during advanced age. Studies have established that the aging brain displays impairment of the NA system (e.g. Goldman-Rakic & Brown, 1981). It is possible that decreased neuronal plasticity during aging may be partially related to a decline in the availability of NA in the brain.

Size of the Lesion. Lashley (1929) demonstrated that there is a direct relationship between cortical lesion size and subsequent behavioral outcome. That is, the larger the lesion the greater the behavioral loss. Although this general rule still holds, recent studies show that it is much too simplistic.

Complete neocortical ablation in adult rats precludes much recovery of function (Whishaw, 1990). In addition, neonatal decortication allows virtually no sparing of function, regardless of the age at surgery (Kolb & Whishaw, 1981a). This suggests that residual cortex may be necessary, but not sufficient, for the Kennard effect to occur. A more recent study (Whishaw & Kolb, 1989) showed that the mere presence of residual cortex in neonatally decorticated rats is not sufficient for sparing of function: Either the amount or the location of the remaining tissue must be important.

Kolb and Tomie (1988) found that neonatal hemidecortication allows significant sparing of certain behavioral functions that is correlated with an increase in cortical thickness in the remaining hemisphere. The behavioral sparing and enhanced cortical thickness are, however, dependent on the integrity of the remaining hemisphere. When animals were hemidecorticated at birth, a small stab wound to the contralateral hemisphere prevented the behavioral sparing and increase in cortical thickness in the remaining hemisphere that is normally seen after hemidecortication (Kolb, 1992).

Clinically, left-hemidecorticated children develop simple language skills because speech shifts to the right hemisphere. However, in children with perinatal injuries to the speech zones of the left hemisphere, lesions of the right hemisphere appear to block the shift of speech from the left to the right hemisphere, resulting in severe and persisting language deficits (Vargha-

Khadem, Watters, & O'Gorman, 1985). For example, one case study describes a boy with dysphasia that was caused by a birth injury to Broca's area (Vargha-Khadem et al., 1985). Early injury to Broca's area usually results in language functions moving to the contralateral hemisphere, but in this case, a lesion in the right precentral region prevented migration of speech to the right hemisphere. This case indicates that communication between various cortical zones may be essential for the development of compensatory functions.

Another aspect of lesion size involves unilateral versus bilateral injury. Hicks and D'Amato (1970) found that sparing of motor behaviors in rats with unilateral motor cortex lesions appears to be correlated with the presence of an abnormal ipsilateral cortico-spinal projection from the normal hemisphere. In contrast however, bilateral cortical injuries may be associated with extensive abnormal connections that are often negatively correlated with behavioral outcome: The animals with the most abnormal connections have the poorest behavioral outcomes (Kolb, Gibb, & van der Kooy, 1994).

There appears to be better recovery of function if part of a functional system is spared by cortical injury. Kolb and Gibb (1990) found that rats with neonatal prefrontal lesions exhibited sparing of many behaviors only if a portion of the prefrontal cortex was left undamaged. Anatomically, reactive sprouting occurs only when part of an existing neural pathway remains after injury (Nieto-Sampedro & Cotman, 1985).

A final way in which lesion size may be associated with recovery is based on observations that relatively small lesions can have more devastating consequences than larger injuries (Irlé, 1987). One possible explanation is the truism "bad brain is worse than no brain". Thus the presence of abnormal brain

may be associated with worse outcome because it interferes with the normal activity of the remainder of the brain. As an alternative explanation, a small injury to the white matter may functionally disconnect two areas of otherwise normal tissue. This process of severing the connections between areas is known as disconnection and the subsequent behavioral effects are called disconnection syndromes. Disconnection syndromes lead to severe behavioral deficits that may be different from what could be expected if one of the areas was damaged (Geschwind, 1965).

Summary - Environment Factor

Humans as well as laboratory animals may benefit from environmental stimulation both before and after brain injury. Clinical investigations demonstrate that cognitive therapies and other environmental manipulations result in improvement in at least some cognitive abilities in people with head injuries (Sohlberg & Mateer, 1989). Even though such therapies appear to be effective in humans, just as in laboratory animals the influence of environmental stimulation may be task-dependent and interact with factors such as the type of lesion, the person's sex and the age at injury (Kolb & Elliott, 1987). Although therapies presently consist almost entirely of behavioral and re-educational strategies, it is possible that in the future we may be able to augment the brain's own compensatory mechanisms to enhance recovery of function.

The Reactive Change Factor

Mechanisms of plasticity that are active during development persist throughout the lifespan of the organism and contribute to recovery of function following injury or disease and during aging. The spontaneous response of the mature brain to neuronal damage includes alteration in dendritic and/or axonal morphology, replacement of lost synapses, and reorganization of the circuitry.

Dendritic Reaction

It is generally accepted that the principal dendritic reaction to brain injury is the loss of terminal branches in response to the loss of afferents but recent studies show that dendritic growth may be a more common reaction to cortical injury. Kolb and Gibb (1991b) found that prefrontal lesions in adult rats produced an increase in dendritic branching in the parietal cortex: The increase correlated with enhanced behavioral performance. Animals with day 10 lesions had the most extensive dendritic branching and the most impressive behavioral sparing whereas the day 1 animals had the least branching and the worst behavioral performance on the same tasks (Kolb & Gibb, 1991a).

Parallel results have been observed in animals with hemidecortications (Kolb, Gibb, & van der Kooy, 1992), cingulate lesions (Kolb & Whishaw, 1991), and visual cortex lesions (Kolb, Ladowski, Gibb, & Gorny, in press). These studies suggest that changes in dendritic arborization may provide a general mechanism underlying recovery of function following brain injury.

Other studies show that changes in dendritic branching also occur in the aging brain. For example, Hinds and McNelly (1977) reported that dendritic

growth in the olfactory bulb of rats was followed by dendritic regression beyond 27 months of age. Coleman and Flood (1987) observed that in normal human aging, there is a net growth of dendrites in some regions of the hippocampus and cortex between middle age and early old age, followed by a regression of dendrites in late old age. They suggested that aging-related loss of neurons is compensated for by an increase in arborization in the remaining cells.

There are two problems associated with dendritic proliferation and subsequent recovery of behavioral function. First, the process of dendritic proliferation in the injured brain appears to be similar to that found during development when there is an overproduction of neuronal processes followed by elimination of inappropriate connections. If developmental and regenerative processes are both serving the same function, then it could be assumed that the injury response is beneficial. But, dendritic proliferation in the injured brain does not automatically mean that the dendritic connections are functional.

Second, there may be limits to the brain's ability to change. Kolb and Gibb (1991b) argued that if the brain changes in response to injury, it may not be capable of further change in response to other factors such as experience. If this is the case, then it is possible that the injured brain may not be able to respond normally to the neuronal loss that occurs with aging resulting in the development of aging-related degenerative diseases (Schallert, 1983).

Axonal Reaction

Following brain injury, uninjured axons may form new branches that attach to vacant synapses. Cotman and Nadler (1978) chose the term "reactive synaptogenesis" to refer to this injury-induced process of synapse formation.

Cotman and his colleagues carried out detailed studies on the time course of reactive synaptogenesis in the dentate molecular layer following unilateral entorhinal ablation in adult rats (Figure 2). For the first few days, degeneration and debris removal are the most prominent morphological effects of the lesion. Two days after the lesion, fibers and synapses display extensive degeneration which proceeds rapidly for 10 days, then at a slower rate thereafter (Matthews, Cotman, & Lynch, 1976a).

This period of rapid degeneration coincides with an impressive glial reaction. Within the first 2 days after the lesion, astrocytes in the molecular layer atrophy, orient their processes toward the denervated area, and migrate partway into this zone. At the same time, microglia triple their pre-lesion numbers within 4 days (Lynch, Rose, Gall, & Cotman, 1975).

The first signs of reactive growth can be observed 4 days after the lesion (Cotman, Matthews, Taylor, & Lynch, 1973; Nadler, Cotman, & Lynch, 1977) and associational and commissural fibers begin to invade the denervated area (Lynch, Gall, & Cotman, 1977). These reactive fibers reach their final state after about 12 to 15 days. Although some new synapses appear to be formed during the initial stages of fiber growth, the most rapid phase of synaptogenesis occurs between 9 and 30 days post-lesion (Lee, Stanford, Cotman, & Lynch, 1977).

Reactive synaptogenesis obviously serves a positive role in preserving damaged circuits by providing presynaptic input to deafferented neurons, thereby preventing dendritic atrophy associated with denervation. Conversely, reactive synaptogenesis of undamaged axons may interfere with the process of functional recovery by introducing abnormal connections that may compete for available postsynaptic sites with regenerating sprouts from damaged axons.

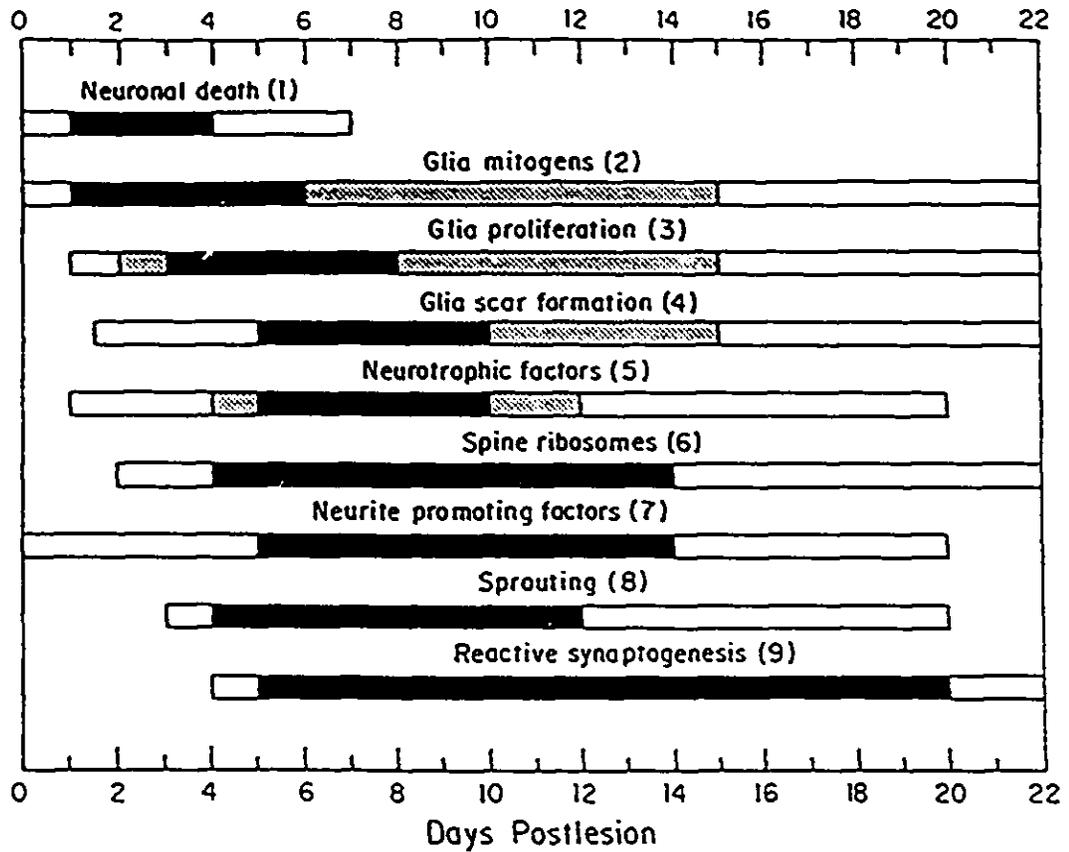


Figure 2. Time course of events following CNS injury. The beginning of the bars indicates when the event is initiated. Many of these events continue beyond the time scale covered in the graph. The intensity of the shading parallels the intensity of the phenomenon indicated above the bar. Note that regeneration promoting responses (4 - 9) occur simultaneously, reaching maxima around 7 - 10 days postlesion. (After Nieto-Sampedro and Cotman, 1985)

Glial Reaction

Injury to the brain causes an increase in the activity of neurotrophic factors in the tissue surrounding the injury and in the wound cavity itself (Nieto-Sampedro et al., 1982; Nieto-Sampedro, Manthorpe, Barbin, Varon, & Cotman, 1983). Two hypotheses were brought forward to account for the cellular source and molecular nature of these factors. The first hypothesis proposed that neurotrophic factors are produced by the target of the disrupted inputs (Cotman, Nieto-Sampedro, & Harris, 1981).

The second hypothesis proposed that neurotrophic and neurite promoting factors are produced by non-neuronal cells and are regulated by neuron-glia interactions (Nieto-Sampedro et al., 1983). This hypothesis was supported by the observation that glial cells proliferate in response to injury with a time course that parallels or slightly precedes an increase in neurotrophic factor activity (Nieto-Sampedro, Saneto, deVellis, & Cotman, 1985). If this hypothesis is correct, then glial cells show a peculiar dichotomous role - they are both beneficial and detrimental. That is, glial proliferation appears to be responsible for trophic and neurite promoting activities but the injury-induced glial boundary, which forms five days after injury (Nieto-Sampedro & Cotman, 1985), prevents regenerating axons from reaching their targets.

When Nieto-Sampedro et al. (1982) demonstrated that brain injury produces a time-dependent increase in neurotrophic activity, they did not determine the identity of these factors. Needels and Cotman (1988), however, found that survival of cultured hippocampal neurons was enhanced when treated with a variety of growth factors, the most effective of which was basic fibroblast growth factor (bFGF).

Basic FGF, a neurotrophic factor synthesized in the central nervous system (CNS), has multiple effects on neurons and glia. This factor has potent effects on the survival and proliferation of glial cells *in vitro* (Eccleston & Silberberg, 1985; Pruss, Bartlett, Gavrilovic, Lisak, & Rattray, 1982) as well as on the survival and outgrowth of neurons *in vitro* (Morrison, Sharma, deVellis, & Bradshaw, 1986; Walicke, Cowan, Ueno, Baird, & Guillemin, 1986) and *in vivo* (Anderson, Dam, Lee, & Cotman, 1988; Otto, Frotscher, & Unsicker, 1989; Sievers, Hausmann, Unsicker, & Berry, 1987).

Gomez-Pinilla, Lee, and Cotman (1992) examined the possibility that bFGF might increase as a result of brain injury. Following unilateral entorhinal cortex lesions in adult rats, the hippocampus ipsilateral to the lesion showed an enhancement of bFGF immunoreactivity in the dentate gyrus outer molecular layer. An increase in the number of bFGF immunoreactive astrocytes was evident on post-lesion day 2, reached a maximum by post-lesion day 7, and decreased to about normal levels by post-lesion day 14. In addition, there was an increase in bFGF immunoreactivity within individual astrocytes in the same area reaching statistical significance by post-lesion day 7 and remaining through post-lesion day 14. The extracellular matrix of the dentate gyrus ipsilateral to the lesion showed an increase in bFGF immunoreactivity with a similar time course. Astrocytes surrounding the lesion also showed increased bFGF immunoreactivity. The results of this study suggest that in response to degenerative events, bFGF is released from astrocytes and becomes available to local neurons and may aid in cell survival and regeneration.

The induction of neurotrophic factors seems to serve a beneficial purpose in the regeneration process but since neuronal death secondary to the

lesion is essentially complete 4 days post-lesion (Nieto-Sampedro & Cotman, 1985) and neurotrophic factors peak 10 days after the injury (Nieto-Sampedro et al., 1983). trophic activity is not sufficiently rapid to provide adequate support to a large proportion of injured neurons (Figure 2). Nieto-Sampedro et al. (1983) hypothesized that neurotrophic factors could prevent secondary cell death if they could be provided early in the regeneration sequence.

For example, Cummings, Yee, and Cotman (1992) showed that unilateral perforant path axotomy (knife cut of the pathway connecting the entorhinal cortex and subiculum with the hippocampal formation) in mature rats caused the death of approximately 30% of the stellate neurons in layer II of the entorhinal cortex within two weeks. Continuous intraventricular infusion of bFGF over a period of 14 days reduced the cell loss to less than 5%.

In a related study, chronic infusion of bFGF following fimbria-fornix transection in adult rats appeared to preserve nerve growth factor (NGF) receptors on neurons within the medial septal complex and prevent the death of medial septal neurons (Gomez-Pinilla et al., 1992). The results of these studies (Cummings et al., 1992; Gomez-Pinilla et al., 1992) indicate that exogenously supplied bFGF may be beneficial as a treatment following brain injury.

Summary - Reactive Change Factor

It appears that injury to the brain provokes a reactive gliosis in which astrocytes proliferate and undergo morphologic changes. These astrocytes actively produce neurotrophic factors which influence neuronal function and morphology. Following brain injury one of these factors, bFGF, appears to directly or indirectly promote neuronal survival and regeneration.

RESEARCH APPROACH

The literature review shows that three factors (age, environment, and reactive change) contribute to the compensatory mechanisms that underlie recovery of behavioral function. The research undertaken in this thesis examined the interactions of these factors. Specifically, I investigated the role of bFGF in brain plasticity and recovery of behavioral function following cortical injury in adult rats.

Finkelstein et al. (1988) observed an increase in bFGF immunoreactivity seven days after focal injury to the dorsolateral frontal cortex of adult rats. The reaction was localized to cells that resemble reactive astroglia in the tissue surrounding the lesion. Experiment 1 was performed to determine whether there is a time-related difference in the expression of bFGF following injury to the motor cortex. Adult rats were given unilateral motor cortex lesions and were sacrificed at different post-lesion times. Brains were processed immunohistochemically and alternate sections were stained for bFGF or glial fibrillary acidic protein (GFAP, a marker for identifying astrocytes).

Many studies have been conducted that suggest a neurotrophic role for endogenous bFGF following brain injury (e.g., Finkelstein et al., 1988; Logan, Frautschy, Gonzalez, & Baird, 1992; Takami et al., 1992) but experiments have not been conducted that would determine the consequences of neutralization of bFGF in the injured brain. The purpose of Experiment 2 was to investigate (a) whether blocking the expression of bFGF affects recovery of function following cortical injury, and (b) whether there is a relationship between recovery of function and neuronal morphology. Pretrained adult rats were given unilateral

motor cortex lesions and one group of rats had anti-bFGF antibodies placed in the lesions. Post-lesion behavior was analyzed using a test sensitive to motor cortex damage. Brains were processed using the Golgi-Cox method and dendritic structure was analyzed.

Studies have shown that glial cells produce neurotrophic and neurite promoting factors (Nieto-Sampedro et al., 1983) and that environmental stimulation induces an increase in the number of glial cells in rat brains (Diamond et al., 1976). Thus, environmentally induced proliferation of glial cells may enhance expression of endogenous bFGF following cortical injury. Experiment 3 examined whether differential environmental housing affects the glia-derived bFGF response to cortical injury. Adult rats were housed in an "enriched" or an "impoverished" environment before and after receiving unilateral motor cortex lesions and were sacrificed at different post-lesion times. Brains were processed immunohistochemically for bFGF and GFAP.

Studies of laboratory animals suggest that some measures of behavioral function can be influenced by environmental factors. For example, Hughes (1965) showed that rats housed in complex environments exhibit reduced behavioral deficits after brain injury when compared to rats housed in impoverished environments. Experiment 4 investigated (a) whether the enhanced astrocytic expression of bFGF induced by pre-lesion environmental enrichment reduces injury-induced behavioral deficits, and (b) whether there is a relationship between behavioral deficits and post-lesion cortical morphology. Adult rats were housed in an "enriched" environment or in standard laboratory cages prior to receiving bilateral frontal cortex ablations. Following recovery, rats were subjected to a battery of behavioral tests sensitive to frontal cortex

lesions. Brains were analyzed for brain weight and cortical thickness.

The results of this research will be relevant to understanding (a) how the brains of adult rats compensate for injury, and (b) whether environmental enrichment plays a role in brain plasticity and recovery of behavioral function following brain injury. As well, the results may have implications for humans with brain injuries. Post-lesion enrichment is often described as post-traumatic "therapy" and pre-lesion enrichment is labelled "protection" from the effects of brain trauma (Stein, Finger, & Hart, 1983). The research undertaken in this thesis could indicate whether "protection" is as essential to recovery from brain damage as is "therapy".

EXPERIMENT 1

Introduction

Focal injury to the brain results in a complex cascade of cellular and molecular events that occurs in the days and weeks following the lesion. Cells located close to the lesion die immediately (primary neuronal death) and cells indirectly affected by the injury die around four days later (secondary neuronal death). During the first few days, repaired blood vessels restore circulation and necrotic tissue is phagocytosed by activated glia and macrophages. Two days after the lesion the glial cell proliferation begins. A new vascularized glial boundary surrounding the lesion site can be observed five days after the injury. Reactive axonal sprouts innervate deafferented cells and regenerative axonal sprouts begin to form eight to ten days after the lesion (Nieto-Sampedro & Cotman, 1985). These events contribute not only to the repair of damaged tissue, but may also contribute to recovery of function following brain injury.

Several peptide growth factors have been implicated as regulators of the injury response: *In vivo* (Cummings, Yee, & Cotman, 1992) and *in vitro* (Needels & Cotman, 1988) studies strongly suggest a role for bFGF as a regulator of the response. If bFGF does play a major regulatory role following brain injury, then it could be predicted that there would be an elevation of its expression at the lesion site.

In this experiment, I characterized the response of bFGF to a precisely defined, focal motor cortex lesion. Specifically, I examined the post-lesion time course of bFGF changes in the brains of adult rats.

Methods and Procedures

Animals

The study was done with 18 three month old male rats, derived from Charles River Long-Evans strains. The animals were housed in groups of 4 - 8 on a 12-hr light/dark cycle at a temperature of 20 - 22° C and were maintained on *ad libitum* food and water.

Surgical Procedures

The rats were anaesthetized with intraperitoneal (ip) injections of sodium pentobarbital (65 mg/kg,) and atropine methyl nitrate (5 mg/kg). Following craniotomy and incision of the dura, focal unilateral suction lesions (4 mm long x 2 mm wide) were made in Zilles' (1985) area FL in the right motor cortex using gentle aspiration through a glass pipette. After surgery, the rats were allowed to survive for 2, 7, 14, or 21 days and the brains were processed for histology.

Rats with lesions (n = 14) and intact control rats (n = 4) were given an overdose of sodium pentobarbital (100 mg/kg, ip) and then intracardially perfused with 250 ml of 0.1 M phosphate buffered saline, pH 7.6 (PBS), followed by 250 ml of 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer, pH 7.6 (PB). The brains were removed, postfixed for 24 hours in fresh solution of the same fixative, then stored for 3 days in 30% sucrose in PB. Coronal sections (50 µm) from pre-frontal cortex, motor cortex, anterior hippocampus, and visual cortex were cut using a Vibratome and placed into 0.1 M PB and were processed for immunoreactivity as described below.

Immunohistochemistry

Sections were incubated in 3% normal serum and 0.3% Triton X-100 in 0.1 M PB, pH 7.6 (TPB) for 20 hours at 2°C to block non-specific binding.

Alternate sections were incubated in rabbit anti-Fibroblast Growth Factor (FGF) - Basic primary antibody (Sigma F-3393) at a concentration of 2.5 μ l/ml, diluted in 3% normal serum in TPB for 60 minutes at room temperature; or were incubated in mouse anti-Glial Fibrillary Acidic Protein (GFAP) primary antibody (Sigma G-3893) at a concentration of 2.5 μ l/ml, diluted in 3% normal serum in TPB for 60 minutes at room temperature. The presence of bFGF was examined with a Vectastain *Elite* Rabbit ABC Kit (Vector PK-6101) and the presence of GFAP was examined with a Vectastain *Elite* Mouse ABC Kit (PK-6102). The sections were rinsed (3 x 3 min) in PB and incubated in biotinylated goat anti-rabbit IgG (Sigma PK-6101) at a concentration of 5 μ l/ml (for bFGF staining) or biotinylated horse anti-mouse IgG (Sigma PK-6102) at a concentration of 5 μ l/ml (for GFAP staining) for 30 minutes at room temperature. The sections were rinsed (3 x 3 min) in PB and incubated in Avidin DH: biotinylated horseradish peroxidase H complex for 30 minutes at room temperature. The sections were rinsed (3 x 3 min) in PB and incubated in a 4% 3,3'-diaminobenzidine (DAB) solution containing 2% hydrogen peroxide (Vector DAB Substrate Kit for Peroxidase SK-4100) for 5 minutes at room temperature. The sections were rinsed (2 x 3 min) in PB and mounted out of 1:1 PB:distilled water onto 1% gelatinized slides, then cleared in Hemo De and coverslipped.

Quantitative Analysis

Sections were viewed using a modified Zeiss microscope with a Heidenhain VRZ 401 microcator (BICO/AS, Glastrup, Denmark). A video camera, connected to a Commodore 10848 monitor, was mounted on top of the microscope. An Amiga 2000 computer equipped with Grid v1.2 Stereology Software (Medico Soft, Silkeborg, Denmark) was connected to the monitor.

Counting frames were superimposed upon the image of the tissue

sections viewed on the monitor. Each counting frame at tissue level occupied an area 300 μm x 200 μm . Cells were counted at a magnification of 100x.

All bFGF-positive astrocytes within two counting frames medial to the lesion were counted and the mean number of bFGF-reactive astrocytes was calculated for each subject. The same counting procedure was followed for GFAP-reactive cells.

Results

Distribution of bFGF in intact rat brain

In intact brains, immunostaining with antisera to bFGF produced prominent staining of several neural structures including hippocampus, amygdala, cingulate cortex, and piriform cortex. Other brain regions prominently stained included septal nuclei, hypothalamus, olfactory tubercle, nucleus of the stria terminalis, habenula, ventromedial caudate, and anterior thalamic nuclei.

At the microscopic level, staining was found primarily in neuronal cell bodies. In addition, in some brain regions (especially hippocampus), staining was also found in small scattered cells with a non-neuronal morphology.

Response of bFGF-reactive cells to motor cortex injury

In injured brains, there was an increase of bFGF immunoreactivity around the cortical lesions starting at post-lesion day 2. This increase in bFGF immunoreactivity was due to the dense accumulation of small bFGF-reactive cells surrounding the area of injury. Immunohistochemistry with anti-GFAP (a marker for identifying astrocytes) and anti-bFGF antibodies on alternate sections clearly identified these cells as astrocytes (Appendix 1).

The lesion effects on bFGF immunoreactivity were expressed as an increase in the number of bFGF-reactive astrocytes over time. The increase in the number of astrocytes was evident at post-lesion day 2, reached a maximum by post-lesion day 7, and remained elevated to post-lesion day 21 (Figures 3 & 4). ANOVA showed significant differences in bFGF reactivity between controls and operates, $F(4,13) = 42.12$, $p < .0001$. Post hoc tests (Fisher PLSD, $p < .05$) showed that the number of bFGF-reactive astrocytes in control rats was significantly different from the number observed on all post-lesion days. The number of bFGF-reactive astrocytes expressed on post-lesion day 2 was significantly different from the numbers expressed on post-lesion days 7, 14, and 21. Numbers of astrocytes expressing bFGF between post-lesion days 7, 14, and 21 were not significantly different.

Response of GFAP-reactive cells to motor cortex injury

Alternate serial sections stained for GFAP immunohistochemistry also showed an increase in the number of astrocytes surrounding the lesion site with a time course similar to that observed with antisera to bFGF. The lesion effects on GFAP immunoreactivity were, like bFGF effects, expressed as a relative increase in the number of GFAP-reactive astrocytes as compared to untreated controls. The increase in the number of astrocytes was evident at post-lesion day 2, reached a maximum by post-lesion day 7, and decreased to about normal levels by post-lesion day 21 (Figure 4). ANOVA showed significant differences in GFAP reactivity between controls and operates, $F(4,13) = 94.7$, $p < .0001$. Post hoc tests (Fisher PLSD, $p < .05$) showed that the number of GFAP-reactive astrocytes in control rats was significantly different from the numbers observed on post-lesion days 7, 14, and 21. GFAP reactivity was significantly different on all post-lesion days except between days 14 and 21.

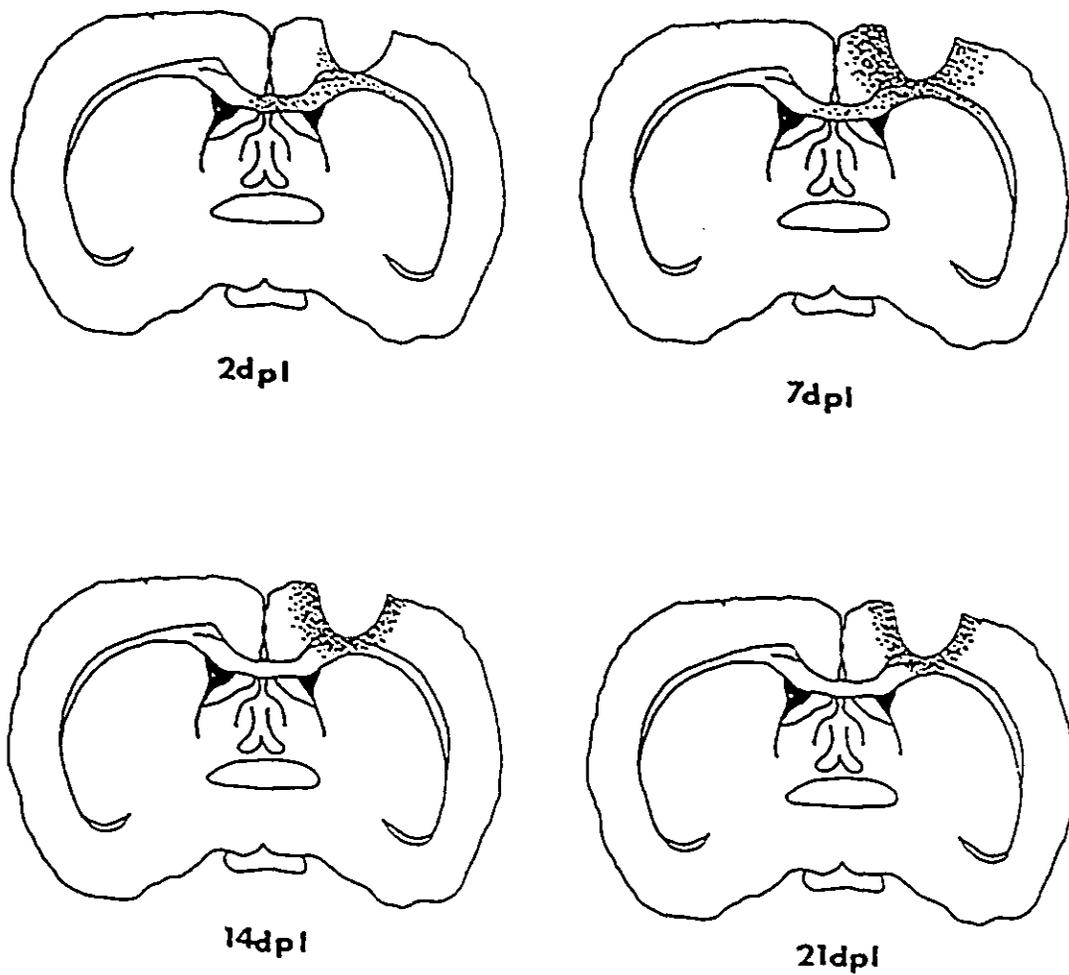


Figure 3. Drawings of coronal sections in representative brains of adult rats with unilateral motor cortex lesions. The sections are located 0.30 mm posterior to bregma (Paxinos & Watson, 1986). Dots represent the distribution of bFGF-reactive astrocytes in the tissue surrounding the lesion. **dpi**, days post-lesion.

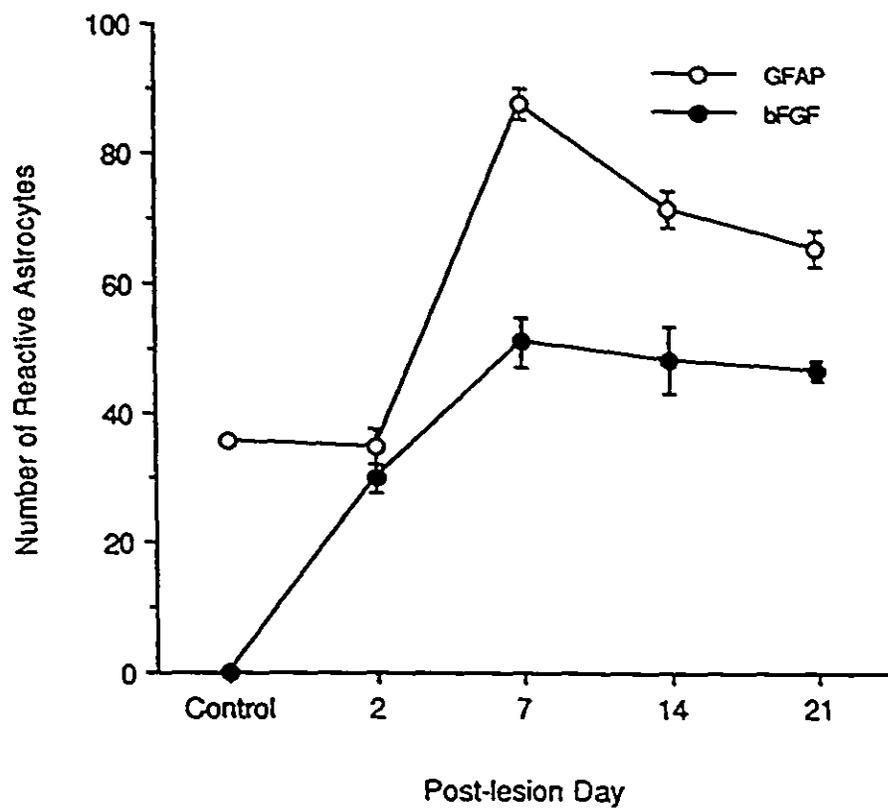


Figure 4. Mean number of bFGF-reactive and GFAP-reactive astrocytes in an area 300 μm long x 200 μm wide x 20 μm thick located medial to unilateral motor cortex lesions. Points represent the mean number of reactive astrocytes; vertical lines depict standard errors of the means.

Discussion

This experiment provides evidence that there is a spatial and temporal change in bFGF reactivity after focal motor cortex injury. Thus, there is an increase in the number of bFGF-reactive astrocytes in the tissue surrounding the lesion two days after injury and this astrocytic response reaches a maximum seven days after injury and then declines. The time course of the astrocytic expression of bFGF is similar to that found by Gomez-Pinilla et al. (1992) in the tissue surrounding entorhinal cortex lesions.

The increased expression of bFGF that occurs seven days after brain injury may be related to neuritic outgrowth that occurs at later times following injury. The peak in astrocyte immunoreactivity for bFGF precedes the formation of reactive and regenerative neuronal sprouts by one to three days (Nieto-Sampedro & Cotman, 1985). The delay in neural sprouting may be due, in part, to the necessity for locally expressed bFGF to be retrogradely transported to neuronal cell bodies before sprouting can occur.

Since brain injury results in a recruitment of bFGF-reactive astrocytes around the lesion one could conclude that bFGF may be an important component in the cascade of regenerative events that occurs after focal brain injury. This raises the question of what role bFGF might play in morphological and behavioral recovery from brain injury.

EXPERIMENT 2

Introduction

Recent studies provide evidence for a neurotrophic role for endogenous bFGF after brain injury. For example, a number of experiments have demonstrated increased bFGF immunoreactivity in the chemically- (Riva, Gale, & Mocchi, 1992) and mechanically- (Finkelstein et al., 1988; Logan et al., 1992; Takami et al., 1992) injured brain. This increase in endogenous bFGF following brain injury is thought to play an important role in wound healing.

In vivo experiments demonstrate that exogenous bFGF also plays a neurotrophic role in the injured brain. Administration of bFGF prevents cholinergic neuron death after fimbria-fornix transection in rats (Anderson et al., 1988; Cummings et al., 1992; Gomez-Pinilla et al., 1992; Miyamoto et al., 1993; Otto et al., 1989). Exogenous bFGF also enhances recovery of the nigrostriatal dopaminergic system following infusion of MPTP in mice (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), a chemical that is selectively toxic to dopaminergic neurons in the substantia nigra (Date et al., 1993; Otto & Unsicker, 1990). In addition, Mattson, Murrain, Guthrie, and Kater (1989) showed that bFGF infusion causes a reduction in hippocampal neuronal death after glutamate-induced axotomy in rats and Liu, D'Amore, Mikati, Gatt, and Holmes (1993) showed similar results after kainic acid-induced, seizure-associated brain damage. Taken together, these findings suggest a role for bFGF as a therapeutic agent to ameliorate the effects of brain injury.

Logan and Berry (1993) noted that key experiments have not been

conducted that might establish a role for bFGF in the brain, such as the consequences for neuronal function of endogenous neutralization of bFGF activity in the intact or damaged brain. In a series of experiments related to this concept, Levi-Montalcini and co-workers found that administration of antibodies to NGF leads to an interference with the supply of NGF to dependent neurons and consequently causes neuronal degeneration (Cohen, 1960; Levi-Montalcini & Booker, 1960; Levi-Montalcini & Cohen, 1960). Based on these studies, the first purpose of the present experiment was to investigate whether administration of anti-bFGF antibodies during surgery to brains with motor cortex lesions would neutralize endogenous bFGF and block the lesion-induced increase in bFGF-reactive astrocytes observed in Experiment 1.

The second purpose of this experiment was to examine the postoperative effects of administration of anti-bFGF antibodies to motor cortex lesions on recovery of function in adult rats. Specifically, I preoperatively trained rats in a reaching task and examined the time course of postoperative recovery of reaching ability.

The third purpose of this experiment was to investigate the postoperative effects of administration of anti-bFGF antibodies on neuronal morphology. I concentrated on changes in basilar dendritic arborization and dendritic spine density in layer V pyramidal cells in area Fr2 (Zilles, 1985) 90 days after motor cortex lesions. I chose these anatomical measures for three reasons. First, dendrites represent up to 95% of the receptor surface that a neuron offers for contact with other neurons (Schade & Baxter, 1960) and spines serve to increase the receptive area of dendrites (Peters & Feldman, 1976). Thus, dendrites and spines can be taken as markers of a neuron's ability to receive

and process information (Coleman & Flood, 1988). Second, dendrites and spines are among the most rapidly changeable neuronal elements visible with light microscopy (Coleman & Flood, 1988) and thus represent a convenient probe to look for evidence of synaptic plasticity. Third, previous studies have correlated changes in both dendritic branching (Coleman & Riesen, 1968; Greenough & Volkmar, 1973) and spine density (Connor & Diamond, 1982; Globus et al., 1973) with learning or enriched rearing. Thus, the primary purpose of this experiment was to determine whether there is a relationship between bFGF immunoreactivity, neuronal morphology, and recovery of function in adult rats treated with anti-bFGF antibodies at the time of motor cortex injury.

Methods and Procedures

Animals

The study was done with 23 male rats derived from Charles River Long-Evans strains that were 90 days old when the experiment began. The animals were housed in groups of 4 - 6 on a 12-hr light/dark cycle at a temperature of 20 - 22°C and were allowed *ad libitum* food and water except during periods of food deprivation.

Rats for Immunohistochemistry. One group of rats, (lesion, anti-bFGF-treated; n = 3), was used to assess bFGF and GFAP immunoreactivity at different times following cortical injury. Rats (lesion, no treatment; n = 15) studied in Experiment 1 made up the control group.

Rats for Behavioral and Golgi-Cox Analysis. There were five groups of rats: (a) control (n = 4); (b) lesion, no treatment (n = 4); (c) lesion, anti-bFGF-treated (n = 4); (d) lesion, saline-treated (n = 4); and (e) lesion, anti-goat IgG-treated (n = 4). Anti-goat IgG was used as a treatment because it is the same molecular size and structure as anti-bFGF antibodies and is non-specific to the system being studied.

Surgical Procedures

Rats for Immunohistochemistry. One group of rats (lesion, anti-bFGF-treated; n = 3) was anaesthetized with sodium pentobarbital (65 mg/kg, ip) and atropine methyl nitrate (5 mg/kg, ip). Following craniotomy and incision of the dura, unilateral suction lesions (4mm long x 2 mm wide) were made in Zilles' (1985) area FL in the right motor cortex. A piece of sterile Gelfoam (Upjohn Inc., Kalamazoo, MI) soaked with anti-bFGF antibodies developed in rabbit (Sigma, F-3393) (1:20) was placed in the lesion cavity.

Rats for Behavioral and Golgi-Cox Analyses. Four groups of rats were anaesthetized as above. Following craniotomy and incision of the dura, unilateral suction lesions (4mm long x 2 mm wide) were made Zilles' (1985) area FL in the motor cortex of the hemisphere contralateral to the preferred reaching paw. One group of rats (lesion, no treatment; n = 4) received no further treatment.

After hemostasis in the remaining three groups, a piece of sterile Gelfoam soaked with either (a) anti-bFGF antibodies (1:20), (b) normal saline (0.9%), or (c) biotinylated anti-goat IgG developed in rabbit (Sigma, BA-5000) (1:20) was placed in the lesion cavity.

Behavioral Methods

Forepaw Reaching. The test was based on one devised by Whishaw, O'Connor, and Dunnett (1986). Rats were trained and tested in nine Plexiglass cages (28 cm deep x 20 cm wide x 25 cm high). The front of each cage was constructed of 2 mm bars that were separated from each other by 1 cm, edge to edge. The floor was constructed of wire mesh with openings 1 cm x 1 cm. A tray (5.5 cm deep x 20 cm wide x 1 cm high) containing chick grower food pellets (obtained from a local feed mill) was mounted on the front of each cage.

To obtain food, the rats had to reach through the bars, grasp the food, and retract the paw and food. The trays were mounted on runners and were retracted 0.5 cm from the front bars so that the rats could not simply scrape the food into the cages. The mesh floor ensured that if the rats dropped food, it fell through the mesh and was lost.

Rats were placed on a food deprivation schedule one day prior to the start of training. Each rat was provided with 15 gm of "Laboratory Rodent Diet 5001" daily throughout the training period. Training consisted of placing the animals in the cages 2 hr each day for 14 days, where they were allowed to obtain food by reaching for it through the front bars of the cages.

At the end of training, the rats were tested for reaching ability and assessed for paw preference. Each reaching test lasted 5 minutes. If a rat made a reaching movement in which a forepaw was inserted through the bars of the cage, the movement was scored as a "reach". If a rat obtained a piece of food and consumed it, the reach was scored as "successful". If a rat failed to grasp a piece of food, or grasped and dropped the food, the reach was scored as "unsuccessful". The animals were tested 21, 14, and 7 days prior to surgery.

Reaching performance in the 7 day pre-lesion test was video-taped using a Sony Betamax camera and recorder.

The rats were tested post-lesion using the same scoring criteria as the pre-lesion testing procedure. Post-lesion, the animals were maintained on *ad libitum* food and water except for the 24 hour period preceding weekly testing when they received no food. The first post-lesion test was conducted seven days following surgery, and the rats were tested every seven days thereafter until 84 days following surgery. Reaching performance was video-taped on post-lesion days 7, 28, 56, and 84.

Anatomical Procedures

Immunohistochemistry. Rats that received anti-bFGF treatment lesions to assess bFGF immunoreactivity were allowed to survive for 2, 7, or 14 days and then were perfused using the same procedure as that used in Experiment 1. The brains were processed for bFGF and GFAP immunoreactivity using the same procedures as those used in Experiment 1. Data were analyzed as they were in Experiment 1.

Golgi-Cox Histology. At the conclusion of behavioral testing, the remaining five groups of rats were given an overdose of sodium pentobarbital (100 mg/kg, ip) and were intracardially perfused with 0.9% saline. Brains were removed and immersed whole in 20 ml of Golgi-Cox solution (Glaser & van der Loos, 1981) for 14 days then were placed in a 30% sucrose solution for 2 days.

Prior to sectioning, brains were blocked perpendicular to the midline at the approximate level of the anterior commissure and again through the caudal portion of the occipital cortices. The tissue was blotted dry and mounted on

sectioning stages. Coronal sections (200 μm) were cut using a Vibratome into 6% sucrose solution, mounted on 2% gelatinized slides, and developed using a procedure described by Kolb and McLimans (1986).

Dendritic Branching. In order to be included in the data analysis, the dendritic trees of pyramidal neurons had to fulfill the following criteria: (a) the cell had to be well impregnated and not obscured with stain precipitations, blood vessels, astrocytes, or heavy clusters of dendrites from other cells; and (b) the basilar arborizations had to appear to be largely intact and visible in the plane of section. Cells were chosen from a section 500 μm anterior to the lesion. The basilar arbors of five layer V pyramidal cells per hemisphere from Zilles' (1985) area Fr2 (Fig. 5) were drawn at a magnification of 200x.

Analysis consisted of drawing the cells with the aid of a camera lucida drawing tube, counting each branch segment, and summarizing by branch order using the procedure of Coleman and Riesen (1968). Branch order was determined for basilar dendrites such that branches originating at the cell body were first order; after one bifurcation, second order; and so on. Statistical analyses were performed by averaging across cells per hemisphere.

Spine Density. Spine density was measured from one order 2 oblique dendritic segment and one terminal dendritic segment in each cell following the procedure of Woolley, Gould, Frankfurt, and McEwen (1990). Spine density measures were made from a segment greater than 10 μm in length. The dendrite was traced (1,000x) with the aid of a camera lucida drawing tube, and the exact length of the dendritic segment was calculated. Spine density was expressed as the number of spines per 10 μm of dendrite. Statistical analyses were performed by averaging across cells per hemisphere.

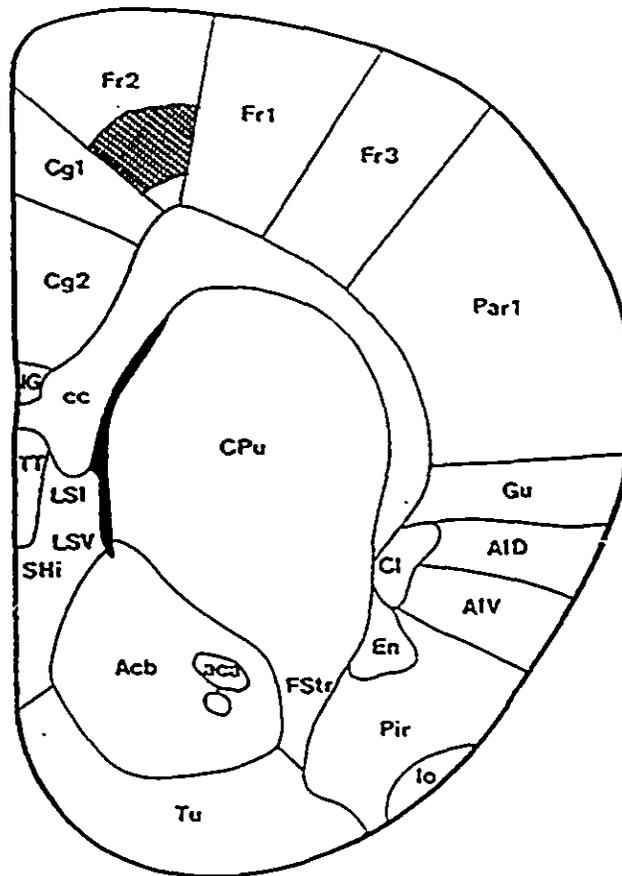


Figure 5. Representation of the right hemisphere of the rat brain 1.7 mm anterior to bregma. The region from which neurons were chosen for dendritic analyses is indicated by the cross-hatched region of area Fr2.

(Abbreviations used in this figure: aca=anterior commissure, anterior; Acb=accumbens nucleus; Al=agranular insular cortex; cc=corpus callosum; Cg=cingulate cortex; Cl=claustrum; CPu=caudate putamen; En=endopiriform nucleus; Fr=frontal cortex; FStr=fundus striati; Gu=gustatory cortex; IG=indusium griseum; lo=lateral olfactory tract; LS=lateral septal nucleus; Par=parietal cortex; Pir=prepiriform cortex; SHi=septohippocampal nucleus; TT=taenia tecta; Tu=olfactory tubercle). (After Zilles, 1985)

Results

Behavioral Results

Forepaw Reaching. Rats with motor cortex lesions in Zilles' (1985) area FL were impaired at reaching for food and made fewer successful reaches than control animals on all post-lesion tests. In addition, rats with lesions that had been treated during surgery with anti-bFGF antibodies were more impaired at reaching than rats that had received either no treatment, treatment with saline, or treatment with anti-goat IgG.

ANOVAs on reaching performance for each post-lesion day indicated that there was no significant difference in the performance of the three groups of lesion rats that had received (a) no treatment, (b) treatment with saline, or (c) treatment with anti-goat IgG. Therefore, I combined the three groups into one group (treatment-control group) in order to increase the power of the statistical analyses.

Figure 6 summarizes reaching behavior over 84 postoperative days and shows three things. First, the reaching behavior of control rats slowly improved over time whereas performance of both groups of rats with lesions improved from post-lesion day 7 to post-lesion day 63. Second, rats with lesions that did not receive anti-bFGF antibodies continued to improve after post-lesion day 63 to almost normal levels by post-lesion day 84. Conversely, rats that had been treated with anti-bFGF antibodies exhibited a decline in reaching performance beginning on post-lesion day 70 that continued through post-lesion day 84. Third, rats treated with anti-bFGF antibodies consistently performed more poorly than both other groups.

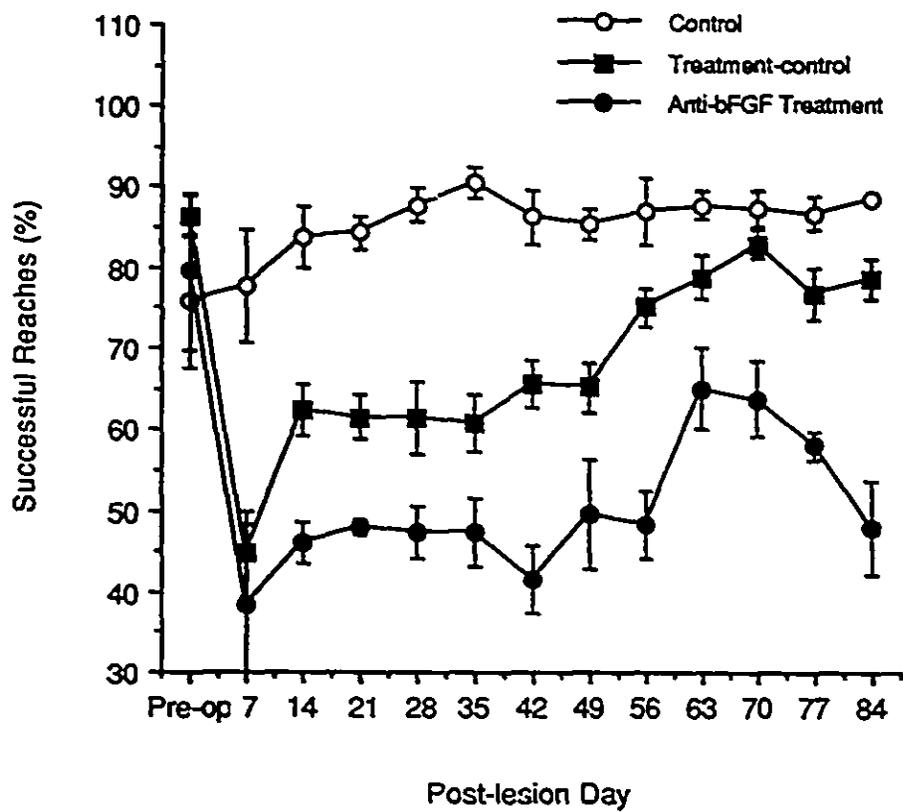


Figure 6. Summary of performance in the Whishaw Reaching Task. Rats were tested weekly following unilateral motor cortex lesions in the hemisphere contralateral to the preferred reaching paw. Points represent the mean percentage of successful reaches; vertical lines depict standard errors of the means.

Two-factor ANOVA on reaching performance over the 12 post-lesion tests showed significant main effects of treatment, $F(2,17) = 41.43$, $p < .0001$; recovery time, $F(11,187) = 16.628$, $p < .0001$; and interaction, $F(22,187) = 2.101$, $p < .0001$. ANOVAs on reaching performance were significant for each post-lesion day: Day 7, $F(2,17) = 6.198$, $p < .0095$; Day 14, $F(2,17) = 16.012$, $p < .0001$; Day 21, $F(2,17) = 21.821$, $p < .0001$; Day 28, $F(2,17) = 10.408$, $p < .0011$; Day 35, $F(2,17) = 17.421$, $p < .0001$; Day 42, $F(2,17) = 24.744$, $p < .0001$, Day 49, $F(2,17) = 12.091$, $p < .0005$; Day 56, $F(2,17) = 22.94$, $p < .0001$; Day 63, $F(2,17) = 6.528$, $p < .0079$; Day 70, $F(2,17) = 16.8$, $p < .0001$; Day 77, $F(2,17) = 10.438$, $p < .0011$; and Day 84, $F(2,17) = 25.579$, $p < .0001$.

Post hoc tests (Fisher PLSD, $p < .05$) showed that on post-lesion day 7 reaching performance of the control group was significantly different than the two groups with lesions. On post-lesion days 14 through 56 the performance of each of the three groups was significantly different from the performance of the other groups on all days. On post-lesion days 63 through 84 the reaching performances of the control group and the treatment-control group were not significantly different whereas the performance of the anti-bFGF group was significantly poorer than the other two groups.

Anatomical Results

Immunohistochemistry. Immunostaining with antisera to bFGF showed that treatment with anti-bFGF antibodies blocked the expression of bFGF in the tissue surrounding the motor cortex lesions. Brains of rats that did not receive anti-bFGF treatment exhibited an increase in bFGF immunoreactivity around the lesions beginning at post-lesion day 2, reaching a

maximum by post-lesion day 7, and remaining elevated through post-lesion day 14 (Fig. 7). Brains of rats treated with anti-bFGF antibodies exhibited a diminished response to injury: bFGF-reactive astrocytes were not visible in the tissue surrounding the lesions on post-lesion day 2, were slightly elevated on post-lesion day 7, and were declining by post-lesion day 14 (Fig. 7).

Two-factor ANOVA on number of bFGF-reactive astrocytes over post-lesion days showed significant main effects of post-lesion day, $F(3,11) = 13.88$, $p < .0005$; treatment, $F(1,11) = 48.34$, $p < .0001$; and interaction, $F(3,11) = 5.96$, $p < .0115$. ANOVAs on number of bFGF-reactive astrocytes were significant for each post-lesion day: Day 2, $F(1,2) = 51.021$, $p < .019$; Day 7, $F(1,3) = 16.589$, $p < .0267$; and Day 14, $F(1,3) = 14.102$, $p < .033$. Post hoc tests (Fisher PLSD, $p < .05$) on post-lesion days 2, 7, and 14 showed that the number of bFGF-reactive astrocytes surrounding cortical lesions was significantly fewer in rats treated with anti-bFGF antibodies than in untreated rats.

Alternate serial sections immunostained with antisera to GFAP showed an increase in the number of astrocytes surrounding the lesion site, quantitatively and temporally similar to the GFAP reaction observed in Experiment 1 (Fig. 8). Two-factor ANOVA on number of GFAP-reactive astrocytes showed significant main effects of post-lesion day, $F(3,11) = 93.006$, $p < .0001$; but not of treatment, $F(1,11) = .175$, $p < .6836$; nor interaction, $F(3,11) = 3.038$, $p < .0747$.

In sum, astrocytes were present in the tissue surrounding motor cortex lesions in a manner temporally and spatially similar to that observed in Experiment 1 but application of anti-bFGF antibodies to the lesions at the time of surgery blocked the astrocytic expression of bFGF.

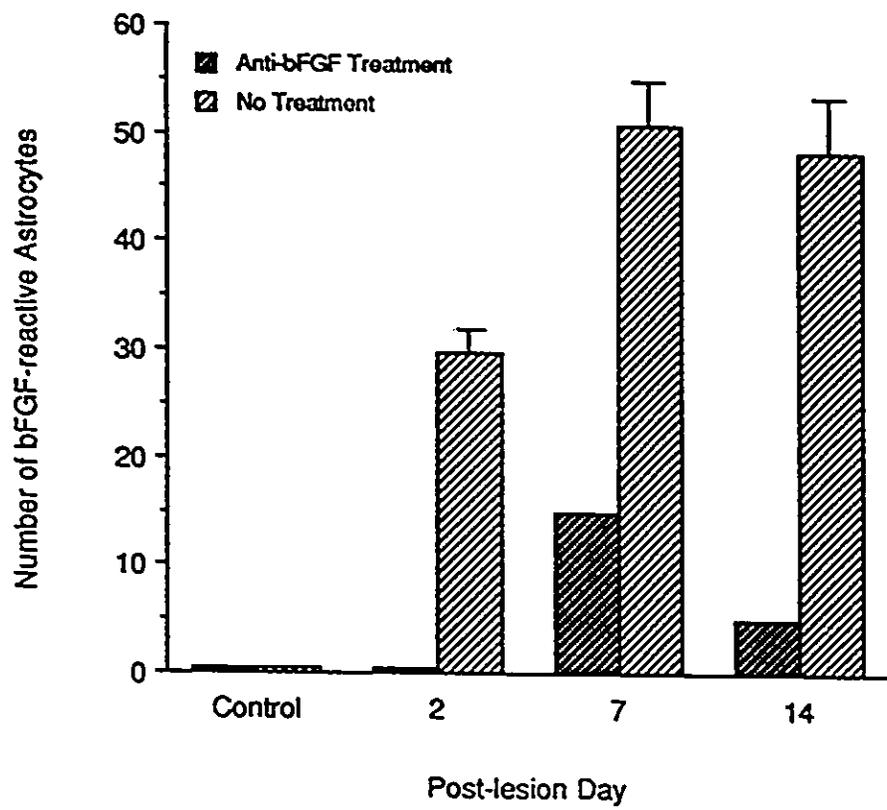


Figure 7. Mean number of bFGF-reactive astrocytes (\pm SE) in an area 300 μ m long x 200 μ m wide x 20 μ m thick located medial to unilateral motor cortex lesions.

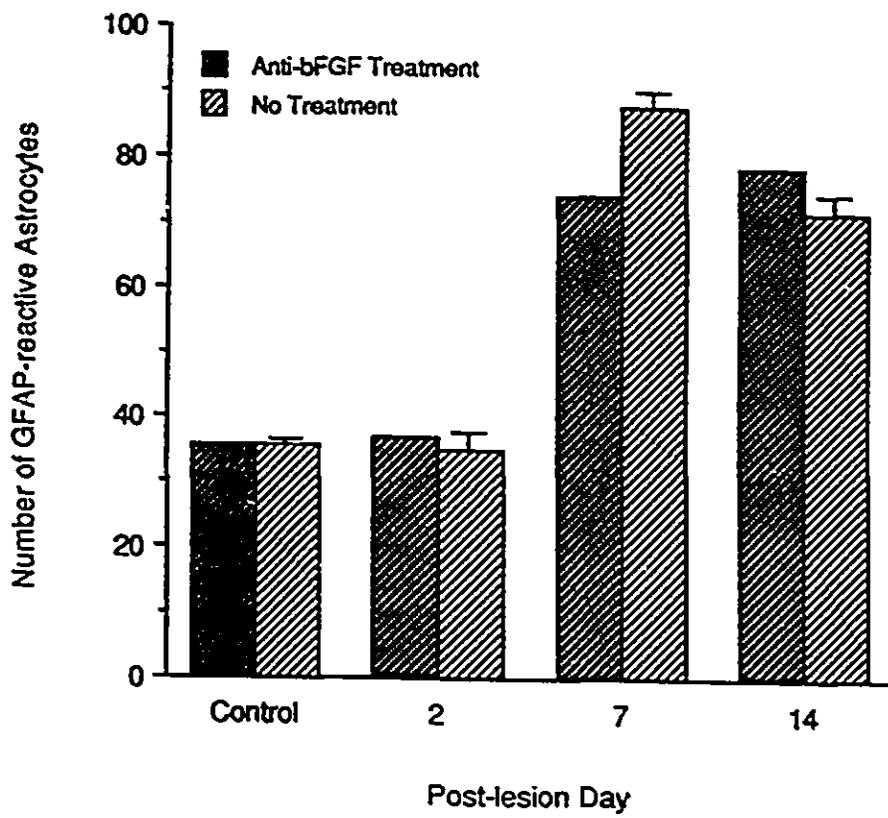


Figure 8. Mean number of GFAP-reactive astrocytes (\pm SE) in an area 300 μ m long x 200 μ m wide x 20 μ m thick located medial to unilateral motor cortex lesions.

Golgi Analysis. When dendritic branching frequency and dendritic spine density were analyzed initially using ANOVAs, there were four obvious results: (a) intact hemispheres of the five groups were virtually identical; (b) intact hemispheres and lesion hemispheres were not significantly different in the control, no treatment, saline-treated, and anti-goat IgG-treated groups; (c) intact hemispheres and lesion hemispheres in the anti-bFGF-treated group were significantly different; and (d) lesion hemispheres in the no treatment, saline-treated, and anti-goat IgG-treated groups were almost identical.

These initial results prompted the following decisions: (a) The no treatment, saline-treated, and anti-goat IgG-treated groups were combined into one group (treatment-control group) in order to increase the power of the statistical analyses; and (b) subsequent analyses considered only lesion hemispheres.

Dendritic Branching. Administration of anti-bFGF antibodies to motor cortex lesions during surgery effected a decrease in dendritic branching in layer V pyramidal cells in Zilles' (1985) area Fr2. Figure 9 shows that anti-bFGF-treated rats had less extensive basilar arborization than the control groups. The total number of branches in control rats and treatment-control rats was very similar. ANOVA on total number of branches showed that there was a significant difference between groups, $F(2,21) = 7.93$, $p < .0027$. Post hoc tests (Fisher PLSD, $p < .05$) showed that the anti-bFGF-treated group had significantly fewer branches than the two control groups, whereas the control and treatment-control groups were not significantly different.

Figure 10 summarizes the dendritic branching frequency in all groups and indicates two clear effects. First, basilar dendritic arborization in control

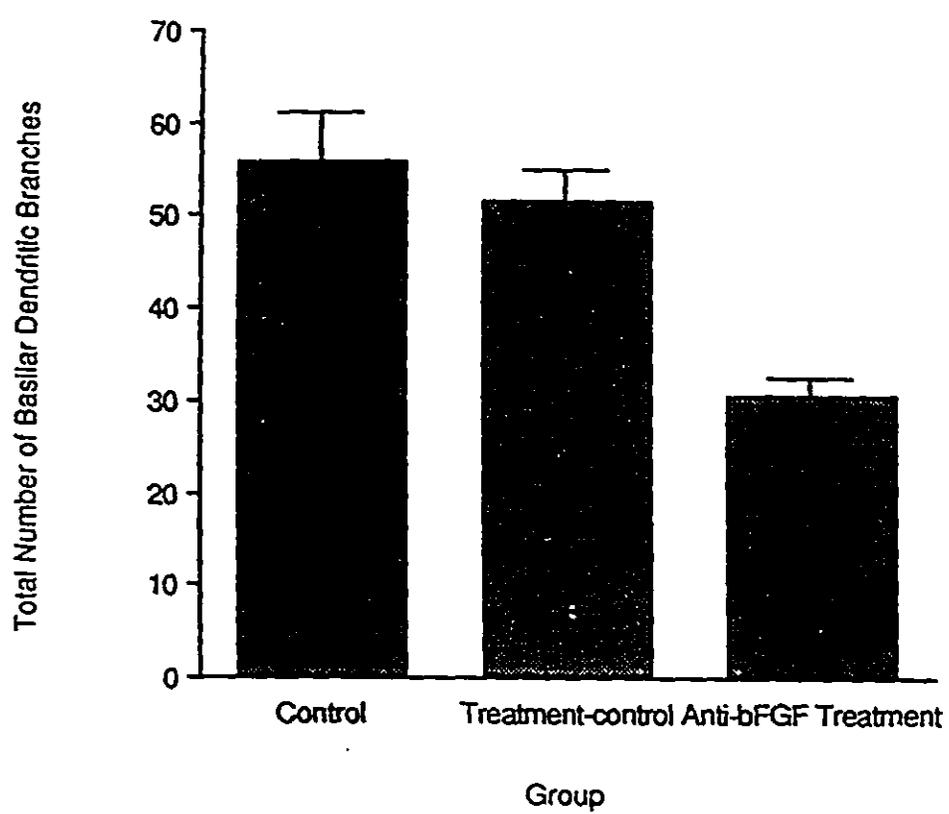


Figure 9. Summary of mean number of total basilar dendritic branches (\pm SE) on layer V pyramidal neurons in Zilles' area Fr2.

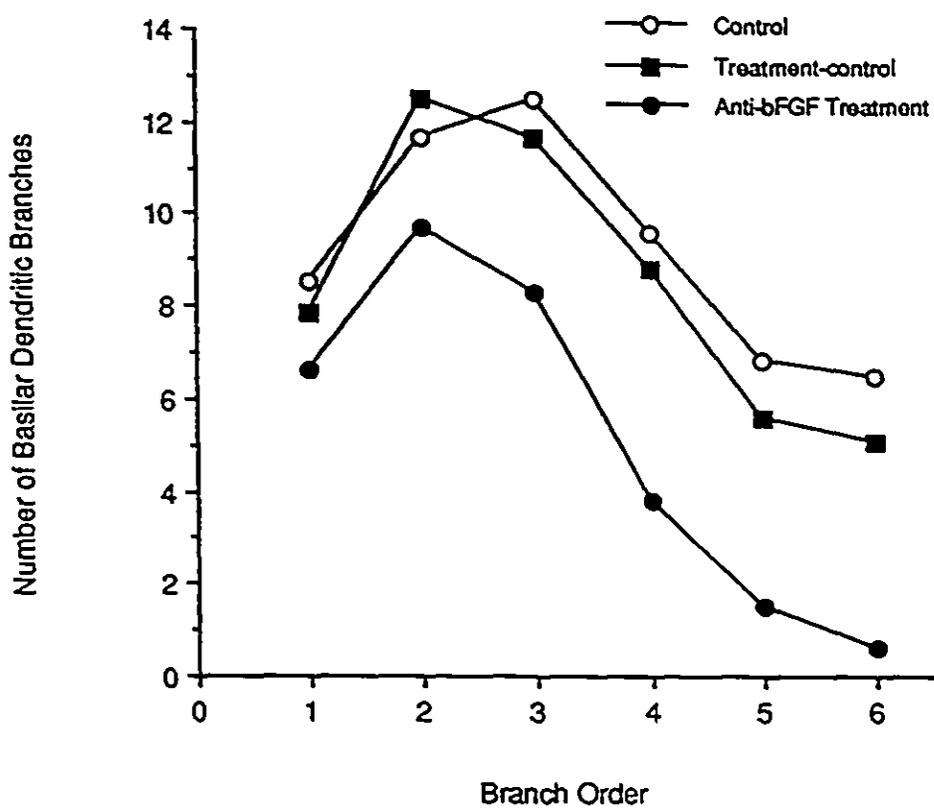


Figure 10. Summary of mean basilar dendritic branching frequency on layer V pyramidal neurons in Zilles' area Fr2 for each branch order. Points represent the mean number of dendritic branches.

rats and treatment-control rats was virtually identical. Second, anti-bFGF-treated rats had less dendritic arborization than the two control groups, particularly in the higher order branches.

Two-factor repeated measures ANOVA on branching frequency across all branch orders showed significant main effects of treatment, $F(2,21) = 7.93$, $p < .0027$; branch order, $F(5,105) = 104.688$, $p < .0001$; and interaction, $F(10,105) = 1.981$, $p < .0426$. ANOVAs on dendritic branching were significant for each branch order: Branch 1, $F(2,21) = 5.17$, $p < .0149$; Branch 2, $F(2,21) = 3.661$, $p < .0433$; Branch 3, $F(2,21) = 3.959$, $p < .0348$; Branch 4, $F(2,21) = 6.758$, $p < .0054$; Branch 5, $F(2,21) = 8.913$, $p < .0016$; and Branch 6, $F(2,21) = 5.451$, $p < .0124$. Post hoc tests (Fisher PLSD, $p < .05$) showed that for all branch orders, the anti-bFGF group had significantly fewer branches than the two control groups, whereas the control and treatment control groups did not differ.

Spine Density. Administration of anti-bFGF antibodies to motor cortex lesions during surgery effected a decrease in basilar dendritic spine density on terminal segments in layer V pyramidal cells in Zilles' (1985) region Fr2. Figure 11 shows that rats treated with anti-bFGF antibodies had fewer dendritic spines per length of terminal branch than did control and treatment-control rats. ANOVA on terminal spine counts was significant, $F(2,21) = 12.814$, $p < .0002$. Post hoc tests (Fisher PLSD, $p < .05$) showed that the anti-bFGF-treated group had significantly fewer terminal dendritic spines than the two control groups.

There were no group differences in spine density on second order oblique branches, $F(2,21) = 3.184$, $p < .062$, although post hoc tests (Fisher PLSD, $p < .05$) showed that the anti-bFGF-treated group had significantly fewer spines than the control group

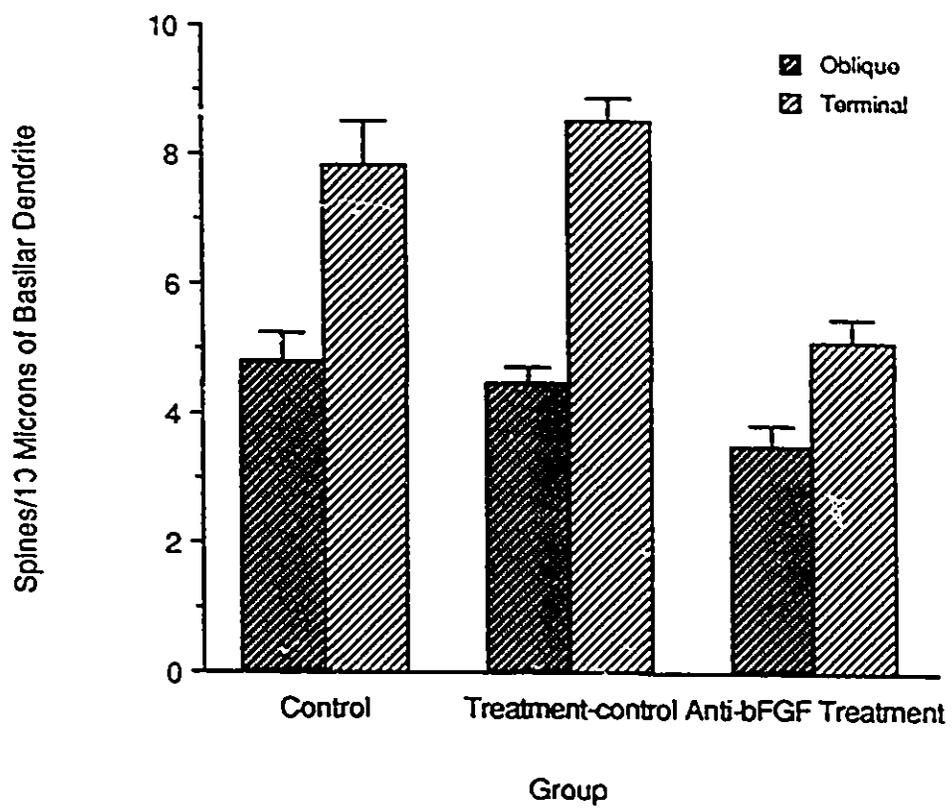


Figure 11. Summary of mean number of spines (\pm SE) on a 10 μ m segment of basilar dendrite on layer V pyramidal neurons in Zilles' area Fr2.

Discussion

The principal results of this experiment are the following: (a) Anti-bFGF antibodies applied to motor cortex lesions during surgery blocked the injury-induced increase of bFGF-reactive astrocytes observed in Experiment 1; (b) rats treated with anti-bFGF antibodies did not recover their pre-lesion levels of reaching ability; and (c) administration of anti-bFGF antibodies resulted in fewer higher order basilar dendritic branches (Fig. 12) and fewer spines (Fig. 13) on terminal dendritic branches. I consider each of these separately.

Astrocytic Response

Gomez-Pinilla et al. (1992) found that bFGF is located in astrocytes and these results were confirmed in Experiment 1: After motor cortex injury there is a temporal and spatial increase in bFGF immunoreactivity in the tissue surrounding the lesion. This response is localized to bFGF-reactive astrocytes that increase in number following injury, peaking on post-lesion day 7. Since brain injury results in a recruitment of bFGF-reactive astrocytes in the tissue surrounding the lesion site one could propose that increased levels of bFGF are necessary to repair damaged cortical tissue and subsequently to enhance recovery of behavioral function.

Basic FGF is considered a potent neurotrophic factor that promotes neuronal survival and neurite extension *in vitro* (Grothe, Otto, & Unsicker, 1989; Walicke, 1988) and *in vivo* (Anderson et al., 1988; Otto et al., 1989; Sievers et al., 1987). Blocking the expression of injury-induced bFGF in the injured brain may interfere with neuronal survival and neuritic outgrowth following injury and ultimately compromise recovery of behavioral function. In fact, the results of

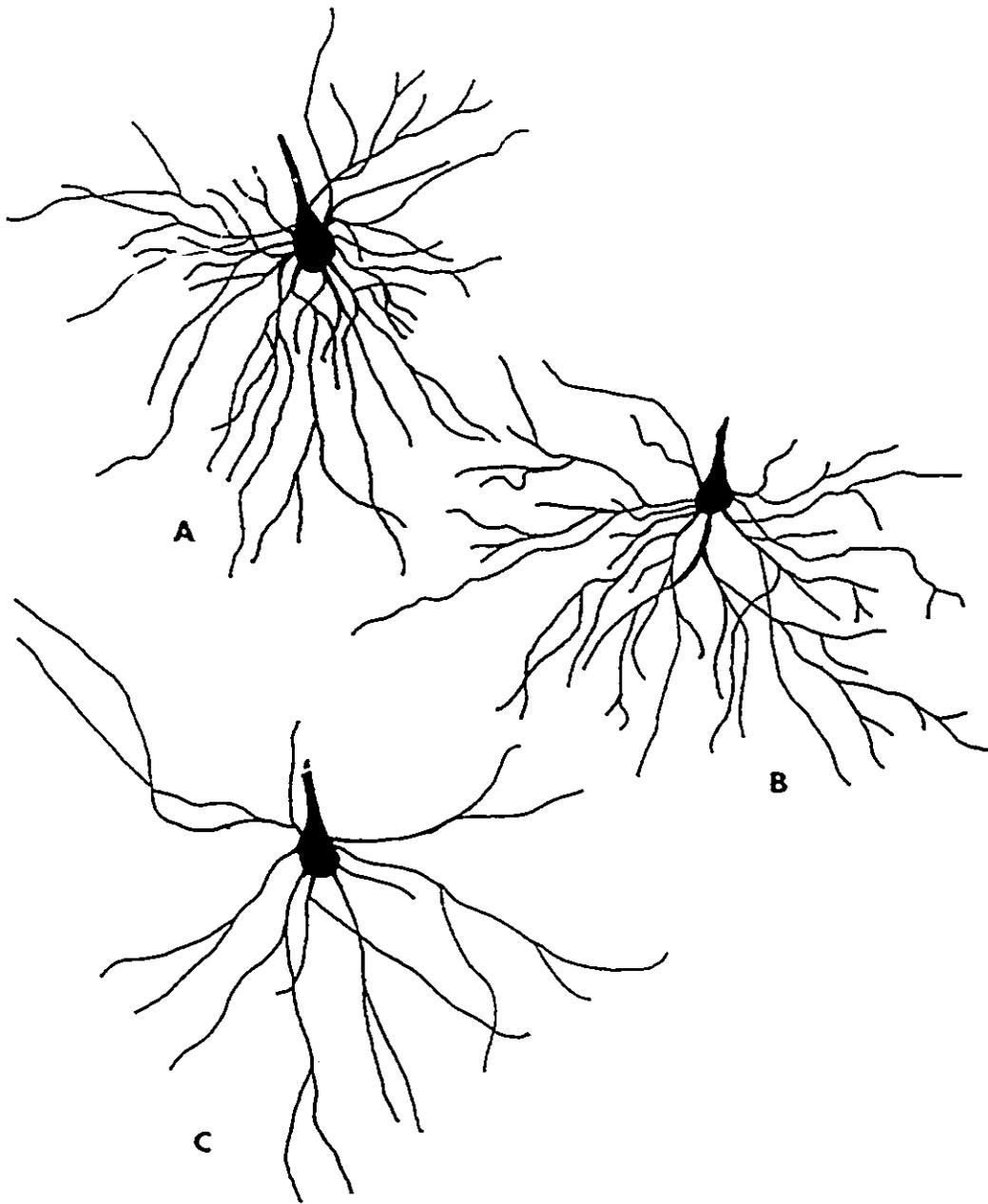


Figure 12. Drawings of the basilar dendritic arbor of representative layer V pyramidal neurons in Zilles' area Fr2. The drawings were made at a magnification of 200x.

A: Control. **B:** Treatment-control. **C:** Anti-bFGF-treated.

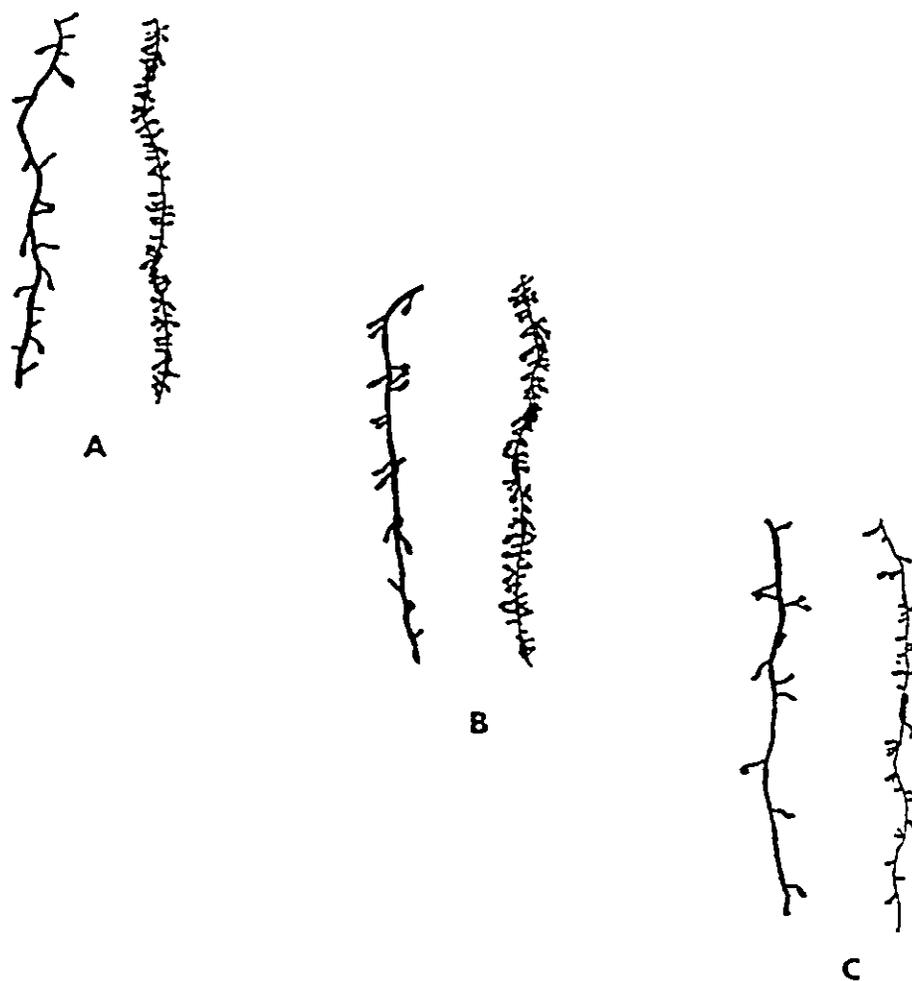


Figure 13. Drawings of basilar dendritic spine density on representative layer V pyramidal neurons in Zilles' area Fr2. The drawings illustrate a segment of a second order oblique dendritic branch (left hand drawing of each pair) versus a segment of terminal branch (right hand drawing of each pair). The drawings were made at a magnification of 1000x.

A: Control. **B:** Treatment-control. **C:** Anti-bFGF-treated.

this experiment indicate that blocking bFGF in the injured brain prevents neuritic outgrowth in layer V pyramidal cells in frontal cortex and precludes recovery of reaching ability following injury.

Behavioral Outcome

Behavior measured immediately following brain injury is different than behavior measured after a period of recovery. Both humans and animals with brain damage typically demonstrate some behavioral recovery: They are most impaired immediately after the injury but the impairments gradually decline over time. In this experiment, reaching ability of all the rats was grossly impaired one week after cortical injury. The treatment-control rats improved to almost normal performance levels after nearly three months recovery time. The reaching ability of animals treated with anti-bFGF antibodies remained impaired for almost two months, at which time reaching performance suddenly improved. The performance levels gradually declined until after three months recovery time, the final performance levels were almost identical to those observed one week after surgery.

These results suggest that blocking the expression of injury-induced bFGF has long-term consequences for behavior. Similarly, the normal injury-induced expression of bFGF may also have long-term effects. It is likely that, even though the expression of bFGF peaks seven days after cortical injury and subsequently declines (see Experiment 1), this immediate response may trigger a cascade of events that is responsible for functional recovery long after bFGF has disappeared from the lesion site. In the absence of the initial bFGF increase the subsequent cascade may be blocked.

Anatomical Substrates

It has been proposed that changes in dendritic structure (dendritic branching frequency and spine density) may reflect a general mechanism of recovery of function after cortical injury (Kolb & Gibb, 1991a, 1991b; Kolb, Gibb, & van der Kooy, 1992; Kolb & Sutherland, 1992). The results of the present experiment are consistent with this general hypothesis. Rats treated with anti-bFGF antibodies showed a decrease in higher order dendritic branching frequency and dendritic spine density (relative to control rats) that was associated with impaired performance in the reaching task. Rats with lesions that were not treated with anti-bFGF antibodies showed no significant differences in dendritic structure nor in reaching performance when compared to control rats.

When the brain is injured, cells located close to the lesion die immediately (primary neuronal death). Cells indirectly affected by the injury die around four days later (secondary neuronal death) (Nieto-Sampedro & Cotman, 1985) possibly as a result of retrograde degeneration following axonal injury. Retrograde degeneration has been attributed to the loss of target-derived neurotrophic factors (one of which is bFGF), which are normally transported along the axon to the cell body (Korsching, 1993). Thus, the assumption could be made that blocking the injury-induced expression of bFGF could result in retrograde degeneration of neurons that are indirectly associated with the damaged area. This assumption is supported by the present experiment: Suppression of bFGF in anti-bFGF treated rats effected neuritic degeneration whereas injury-induced expression of bFGF in the untreated rats prevented neuritic degeneration.

Together, the results of Experiments 1 and 2 suggest that in response to cortical injury, endogenous bFGF is expressed by astrocytes surrounding the lesion site, whereupon it prevents neuritic degeneration and ultimately contributes to recovery of behavioral function. The question arises whether it is possible to enhance the injury-induced astrocytic expression of endogenous bFGF following cortical injury.

EXPERIMENT 3

Introduction

It has been established that exposing rats to an enriched environment results in a constellation of neuroanatomical, neurochemical, and behavioral changes. Environmental stimulation induces increased cortical weight (Diamond et al., 1972) and increased cortical thickness (Diamond, 1967; Diamond et al., 1972), which may be due to neuronal changes such as increased dendritic branching (Greenough & Volkmar, 1973; Greenough, Volkmar, & Juraska, 1973), more dendritic spines (Globus et al., 1973), more synapses per neuron (Turner & Greenough, 1985), and larger synapses (West & Greenough, 1972). In addition, rats housed in an enriched environment exhibit enhanced protein synthesis in the brain (Rosenzweig et al., 1972), and superior performance in complex mazes (Greenough, 1976).

Environmental enrichment influences glial cells as well as neurons. Greater numbers of glial cells were found in the brains of rats housed in an enriched environment when compared to rats housed in isolation (Diamond et al., 1976; Diamond et al., 1966). Sirevag and Greenough (1987) found that astrocytic nuclear volume was greater in rats reared in "environmental complexity" than in rats housed in group lab cages or in individual cages.

The mechanisms mediating these neuronal and glial changes are obscure, but it is reasonable to expect that environmentally-induced morphological changes may be potentiated by growth factors. Will (1981) speculated that nerve growth factor (NGF) might be critically involved in the

mediation of differential housing effects. This speculation received indirect support from a study by Mohammed, Winblad, Ebendal, and Larkfors (1990) who reported that housing rats in different environments results in subtle changes in levels of NGF in certain brain areas.

If an enriched environment induces an increase in glial cells and an elevation of trophic factors in the intact brain, then it could be predicted that environmental stimulation would have a similar effect on the injured brain. Experiment 1 showed that there was a transient increase in bFGF-positive astrocytes around cortical lesions beginning two days after injury. The astrocytic response reached a maximum seven days after injury, then declined suggesting that bFGF may be a crucial component in the brain's response to injury.

This experiment examined whether environmental enrichment is a factor in enhancing the expression of bFGF in the injured brain. Specifically, I characterized the astrocytic response of bFGF to motor cortex lesions in two groups of rats, one group housed in standard laboratory cages and the other group housed in an enriched environment before and after cortical injury.

Methods and Procedures

Animals

The study was done with 23 male rats, derived from Charles River Long-Evans strains. The animals were 75 days of age when the experiment began. There were two main groups of rats (laboratory-housed and enriched-housed), each of which was divided into three sub-groups (control, post-lesion day 7, and post-lesion day 21). The six groups were composed as follows: laboratory-

housed control (n = 4), laboratory-housed post-lesion day 7 (n = 4), laboratory-housed post-lesion day 21 (n = 3), enriched-housed control (n = 4), enriched-housed post-lesion day 7 (n = 4), and enriched-housed post-lesion day 21 (n = 4). Animals were maintained on *ad libitum* food and water. The animal colony was maintained on a 12-hr light/dark cycle at a temperature of 20 - 22°C.

Housing Conditions

The rats were reared with their mothers in 22 x 14 x 18 cm Plexiglass cages until they were 22 days of age then were sexed and housed with same sex rats. At the beginning of the experiment, the laboratory-housed animals were placed in standard 18 x 25 x 20 cm stainless-steel hanging cages in groups of four (except laboratory-housed post-lesion day 21 [n = 3]). The rats remained in these cages for the next 35 days after which time motor cortex ablations were performed on seven of the animals. Following surgery, the animals were returned to their cages.

The enriched-housed animals were placed in two groups of six in two large pens measuring 1.8 m high x 1 m deep x 1.5 m wide. The back wall and floor were made of plywood covered with Arborite, and the remaining walls and roof were covered with hardware cloth. There were various runways and platforms attached to the rear wall so that the animals could sit at different levels. The floor was covered with shredded paper upon which was placed a variety of objects including polyvinyl chloride (PVC) pipes, tree branches, empty cages, tubs, and boxes. The objects were moved daily and replaced weekly. The rats remained in the pens for the next 35 days after which time motor cortex ablations were performed on eight animals (four from each pen). Following surgery, the animals were returned to the pens.

Surgical Procedures

Rats were anaesthetized with sodium pentobarbital (65 mg/kg, ip) and atropine methyl nitrate (5 mg/kg, ip). Following craniotomy and incision of the dura, focal unilateral suction lesions were made in Zilles' (1985) area FL in the right motor cortex using gentle aspiration through a glass pipette. Following surgery, rats were returned to their pens or to their cages.

Following surgery, rats with lesions were allowed to survive for 7 or 21 days after which time the rats were sacrificed and the brains were processed for bFGF and GFAP immunoreactivity. Rats with lesions (n = 15) and intact control rats (n = 8) were given an overdose of sodium pentobarbital (100 mg/kg, ip) and were intracardially perfused with 250 ml of 0.1 M phosphate buffered saline, pH 7.6 (PBS), followed by 250 ml of 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer, pH 7.6 (PB). The brains were removed, postfixed for 24 hours in fresh solution of the same fixative, then stored for three days in 30% sucrose in PB. Coronal sections (50 μ m) at the level of prefrontal cortex, motor cortex, anterior hippocampus, and visual cortex were cut using a Vibratome and placed into 0.1 M PB.

Immunohistochemistry

The immunohistological procedures were the same as those used in Experiment 1.

Quantitative Analysis

Data were analyzed as they were in Experiment 1.

Results

Response of bFGF-reactive cells to motor cortex injury

In intact brains of both laboratory-housed and enriched-housed rats, immunostaining with antisera to bFGF produced a pattern of neuronal staining similar to that observed in intact brains in Experiment 1 (Appendix 1). In injured brains, there was an increase of bFGF-reactive astrocytes around the cortical lesions at post-lesion day 7 continuing through post-lesion day 21 (Figure 14).

The lesion effects on bFGF immunoreactivity were expressed as an increase in the number of bFGF-reactive astrocytes over time. Two-factor ANOVA revealed significant main effects of post-lesion day, $F(2,17) = 486.81$, $p < .0001$; environmental condition, $F(1,17) = 85.06$, $p < .0001$; and interaction of post-lesion day and environmental condition, $F(2,17) = 22.85$, $p < .0001$.

ANOVA revealed significant differences in numbers of bFGF-reactive astrocytes expressed on different post-lesion days for laboratory-housed rats, $F(2,8) = 121.37$, $p < .0001$; and enriched-housed rats, $F(2,9) = 467.14$, $p < .0001$. Post hoc tests (Fisher PLSD, $p < .05$) showed that for both laboratory-housed rats and enriched-housed rats, rats with lesions had significantly more bFGF-reactive astrocytes than control rats.

The increase in bFGF immunoreactivity was more pronounced in enriched-housed rats. They exhibited more bFGF-reactive astrocytes in the tissue surrounding the lesions than did laboratory-housed rats on both post-lesion day 7, $F(1,6) = 20.9$, $p < .0038$; and post-lesion day 21, $F(1,5) = 121.96$, $p < .0001$.

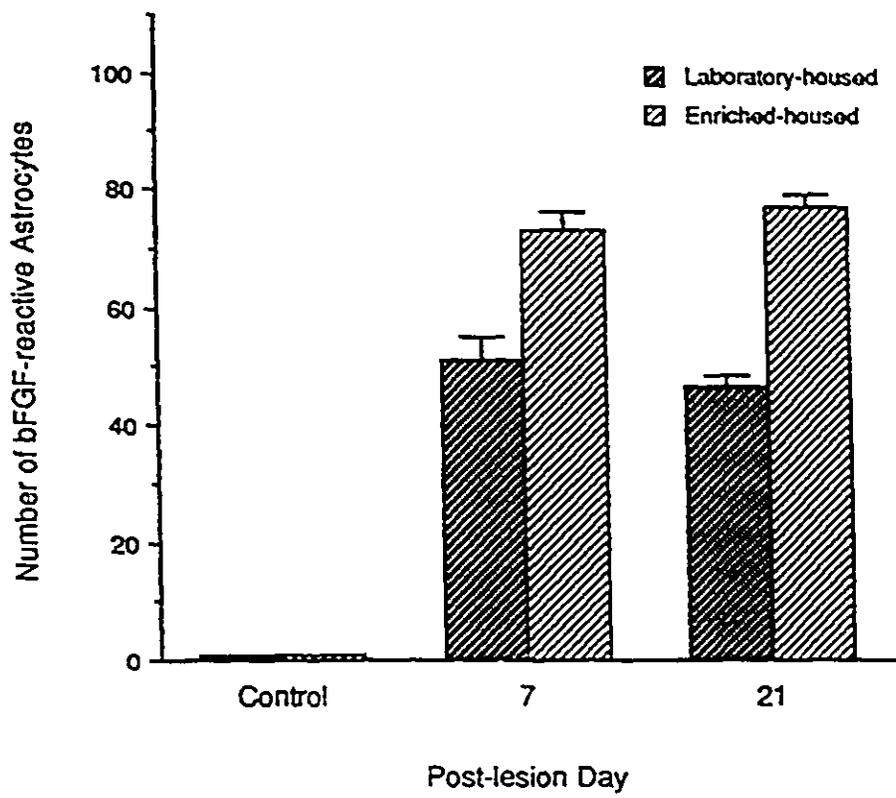


Figure 14. Mean number of bFGF-reactive astrocytes (\pm SE) in an area 300 μ m long x 200 μ m wide x 20 μ m thick located medial to unilateral motor cortex lesions in the brains of laboratory-housed rats and enriched-housed rats.

Response of GFAP-reactive cells to motor cortex injury

Alternate serial sections immunostained with antisera to GFAP showed that in intact and injured brains there was an increase in the number of GFAP-reactive astrocytes around cortical lesions at post-lesion day 7 declining through post-lesion day 21 (Figure 15).

The lesion effects on GFAP immunoreactivity were expressed as an increase in the number of GFAP-reactive astrocytes over time. Two-factor ANOVA revealed significant main effects of post-lesion day, $F(2,12) = 42.42$, $p < .0001$; and environmental condition, $F(1,12) = 37.63$, $p < .0001$; but not interaction of post-lesion day and environmental condition, $F(2,12) = 3.43$, $p < .0663$.

ANOVA revealed significant differences in numbers of GFAP-reactive astrocytes expressed on different post-lesion days for laboratory-housed rats, $F(2,3) = 31.45$, $p < .0097$; and enriched-housed rats, $F(2,9) = 15.88$, $p < .0011$. Post hoc tests (Fisher PLSD, $p < .05$) showed that all groups of laboratory-housed rats were significantly different whereas enriched-housed rats showed significant differences only between controls and both post-lesion day 7 and post-lesion day 21 rats. In enriched-housed rats, the number of GFAP-reactive astrocytes was not significantly different on post-lesion days 7 and 21.

The increase in GFAP immunoreactivity was more pronounced in enriched-housed rats. They exhibited more GFAP-reactive astrocytes around the lesions than did laboratory-housed rats at both post-lesion day 7 and post-lesion day 21. ANOVA showed significant differences in reactivity between lab-housed rats and enriched-housed rats for controls, $F(1,4) = 30.06$, $p < .0054$; and at post-lesion day 21, $F(1,4) = 16.27$, $p < .0157$.

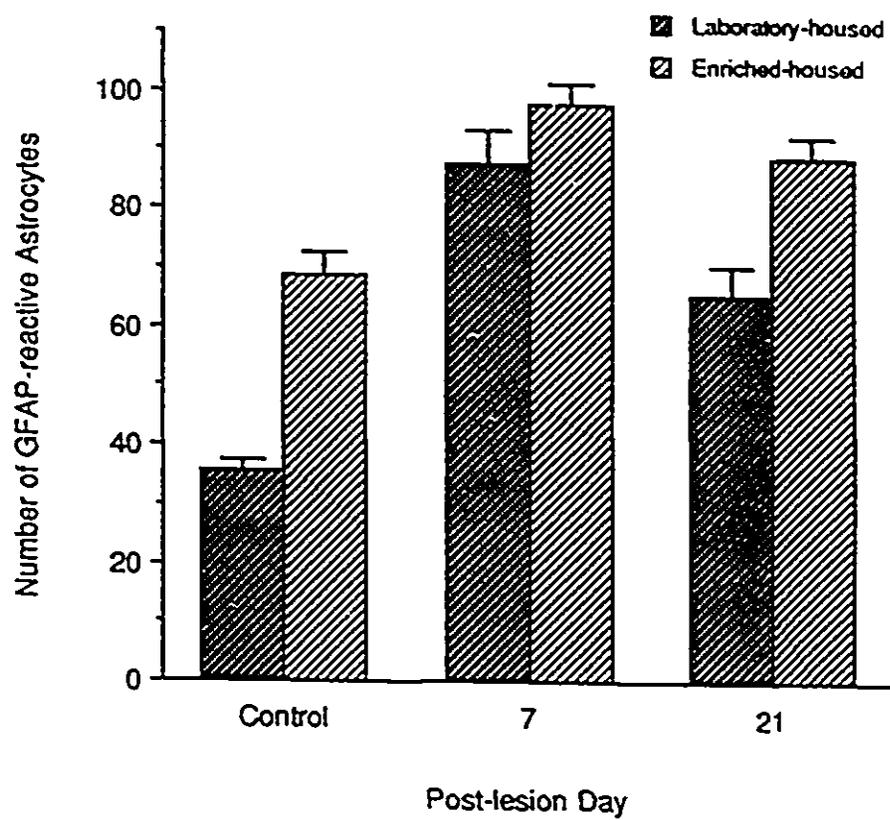


Figure 15. Mean number of GFAP-reactive astrocytes (\pm SE) in an area 300 μ m long x 200 μ m wide x 20 μ m thick located medial to unilateral motor cortex lesions in the brains of laboratory-housed rats and enriched-housed rats.

Discussion

This experiment demonstrates that housing rats in different environments results in variations in injury-induced astrocytic expression of bFGF following brain injury. In both the enriched-housed rats and the laboratory-housed rats, there was an increase in the number of bFGF-reactive astrocytes that closely approximates the temporal and spatial astrocytic changes observed in Experiment 1. Rats that had been housed in an enriched environment however, exhibited an enhanced response to cortical injury as they had more bFGF-reactive astrocytes in the tissue surrounding the lesions than did rats housed in standard laboratory cages. Thus, one could propose that housing rats in an enriched environment may be an effective, non-invasive method of enhancing the astrocytic expression of endogenous bFGF following brain injury.

Enhancing levels of bFGF in the injured brain can also be accomplished by using invasive surgical procedures. For example, intraventricular infusion of exogenous bFGF after fimbria-fornix transection in rats increases cortical levels of bFGF and subsequently enhances neuronal survival (Cummings et al., 1992; Gomez-Pinilla et al., 1992). There are two reasons why introduction of exogenous bFGF may not be the most effective method of enhancing levels of endogenous bFGF in the injured brain. First, there may be time constraints that impact on the effectiveness of exogenous bFGF. A recent study demonstrated that topical application of bFGF to tissue surrounding somatosensory cortex lesions in rats prevented retrograde neuronal degeneration only when applied immediately after injury (Kohmura, Yuguchi, Yamada, Sakaguchi, & Hayakawa, 1994). Thus, exogenous bFGF may be effective only during the early phase of

the regeneration sequence.

Second, a consistently enhanced trophic milieu in the injured brain may be necessary to sustain neuronal survival and neuritic outgrowth (Logan & Berry, 1993). The enhanced expression of endogenous bFGF that is a consequence of environmental enrichment may (a) ensure that elevated levels of bFGF are present during the early stages of the injury response, and (b) provide a constant trophic milieu that facilitates sustained neuronal survival and prevents neuritic degeneration following cortical injury.

Experiment 2 indicated that a lack of endogenous bFGF in the injured brain contributes to neuritic degeneration and precludes recovery of behavioral function following cortical injury. Expression of normal levels of injury-induced bFGF, however, results in post-lesion recovery of function that is associated with dendritic branching frequency and dendritic spine density similar to that observed in intact rats. Thus, it could be predicted that increasing the astrocytic expression of injury-induced bFGF by pre-lesion environmental enrichment will enhance neuritic outgrowth and reduce post-lesion behavioral deficits.

EXPERIMENT 4

Introduction

Environmental factors play an important role in the development and plasticity of brain structure and function. The importance of the interaction between an organism and its environment has been demonstrated by experiments that document environmentally-induced modifications of brains (Diamond et al., 1972; Diamond, 1967) and behavior (Greenough, 1976) of intact animals.

Studies of animals with injured brains show that recovery varies with age, (Kolb, 1987; Kolb & Elliott, 1987), lesion size (Kolb & Whishaw, 1981a, 1985), and behavioral measure (Kolb & Whishaw, 1981b, 1983). The effects of post-lesion environmental enrichment on behavior and brain morphology are also age- (Kolb & Elliott, 1987; Whishaw, Zaborowski, & Kolb, 1984), lesion- (Dalrymple-Alford & Kelche, 1985, 1987; Kelche et al., 1987; Will et al., 1981), and task-specific (Kolb & Gibb, 1991a).

A relationship between pre-lesion environmental enrichment and post-lesion recovery of behavioral function has been demonstrated in experiments in which rats were housed preoperatively in complex environments. The results of two studies (Donovick, Burrig, & Swidler, 1973; Donovan, Burrig, & Bensten, 1975) suggest that there is an environmental effect independent of the behavioral recovery process. In contrast, Hughes (1965) proposed that there is an interaction between pre-lesion experience and post-lesion behavioral recovery.

Basic FGF may mediate the relationship between pre-lesion environmental enrichment and post-lesion recovery of behavioral function. Following cortical injury, astrocytes surrounding the lesion express bFGF (Experiment 1), which is associated with recovery of function (Experiment 2). This functional recovery correlates with an absence of neuritic degeneration (Experiment 2). The injury-induced astrocytic expression of bFGF is enhanced by environmental enrichment (Experiment 3).

This experiment examined the effects of pre-lesion environmental manipulation on injury-induced behavioral deficits and on post-lesion cortical morphology in adult rats. I predicted that enhanced bFGF expression associated with pre-lesion environmental enrichment would (a) reduce post-lesion behavioral deficits, and (b) enhance neuritic outgrowth and thereby increase cortical thickness that would persist after bilateral ablation of the frontal cortex.

Methods and Procedures

Animals

The study was done with 28 male rats, derived from Charles River Long-Evans strains. The animals ranged from 70 days to 114 days of age when the experiment began. There were two main groups of rats (control, frontal operate), each of which was divided into two groups (laboratory-housed, enriched-housed). The four groups were composed as follows: control, laboratory-housed (n = 10); control, enriched-housed (n = 6); frontal, laboratory-housed (n = 6); frontal, enriched-housed (n = 6).

Housing Conditions

Rats were reared with their mothers in 22 x 14 x 18 cm Plexiglass cages until they were 22 days of age then were sexed and housed with same sex rats. At the beginning of the experiment, the lab-housed animals were placed in standard 18 x 25 x 20 cm stainless-steel hanging cages in groups of four. The animal colony was maintained on a 12-hr light/dark cycle at 20-22°C and the rats were maintained on ad libitum food and water except during specified periods of food deprivation. The rats remained in these cages for the next 50 days after which time frontal cortex ablations were performed on six of the animals. The rats with frontal lesions were then housed in two groups of three and the controls were housed in two groups (n = 4; n = 6).

The enriched-housed animals were placed in two groups of six in two large pens (described in Experiment 3). A variety of objects, including polyvinyl chloride (PVC) pipe, tree branches, empty cages and boxes, were placed in the pens. The objects were moved daily and replaced weekly. The animals remained in the pens for the next 50 days after which time frontal cortex ablations were performed on six animals. The enriched-housed animals were then transferred to standard 18 x 25 x 20 cm stainless-steel hanging cages. The animals with lesions were housed in two groups of three and the controls were housed in two groups (n = 2; n = 4). The animals remained in these cages for the duration of the experiment.

Surgical Procedures

The rats were anaesthetized with sodium pentobarbital (65 mg/kg, ip) and atropine methyl nitrate (5 mg/kg, ip). The frontal neocortex of each rat was exposed by removing the skull from the bregmoidal junction anteriorly to the

frontal bone suture, and laterally to the temporal ridge. After retraction of the dura, the exposed neocortex was removed by aspiration. The external capsule was left in place to reduce the chance of inadvertently damaging the underlying caudate putamen. The intent was to remove all of the midline anterior cingulate and infralimbic cortex, and the medial region of Fr 1/2. Following surgery, the rats were placed in standard laboratory cages. The animals were allowed to recover for two weeks before behavioral training and testing began.

At the conclusion of behavioral testing, the rats were given an overdose of sodium pentobarbital (100 mg/kg, ip) and intracardially perfused with a solution of 0.9% saline followed by 10% formal saline. The brains were removed, weighed and placed in 30% sucrose formalin for 48 h before being cut frozen at 40 μ m. Every tenth section was saved and stained with Cresyl violet.

In order to estimate the loss of brain tissue, the brains were weighed immediately following removal from the skull. Before weighing, the spinal cord was cut even with the caudal edge of the cerebellum, the cerebellar paraflocculi were removed, the optic nerves were severed 1-2 mm anterior to the chiasm, the pineal gland was removed, and all remaining dura was stripped off.

Anatomical Analysis

Neocortical thickness was measured by projecting images of the Nissl-stained sections on a Zeiss 2 POL projector set at a magnification of 13x. According to the procedure described elsewhere (Kolb & Whishaw, 1981), measurements were taken at each of three planes (anterior-posterior location) corresponding to Paxinos and Watson's (1986) bregma -1.8, bregma -4.5, and bregma -6.8 and at three places (medial-lateral location) within each plane.

Behavioral Methods

The animals were given tests shown to be sensitive to bilateral frontal cortical injury in the early postoperative period (Kolb, 1984).

Forepaw Reaching. The test was based on one devised by Whishaw et al., (1986). The equipment used for training and testing the rats was the same as that described in Experiment 2.

Rats were placed on a food deprivation schedule one day prior to the start of training. Each rat was provided with 15 gm of "Laboratory Rodent Diet 5001" daily throughout the training and testing period. Training consisted of placing the rats in test cages 2 hr each day for 7 days, where they were allowed to obtain food (chick grower food pellets) by reaching for it through the grids of the cage.

At the end of training, the rats were tested for reaching ability. Each reaching test lasted 10 min. The performance of the rats was video-taped using a Sony Betamax camera and recorder so that reaches could be more easily observed and scored. If a rat made a reaching movement in which a paw was inserted through the bars of the cage, the movement was scored as a "reach". Reaches were further scored as either "successful" or "unsuccessful". If a rat obtained a piece of food and then consumed it, the reach was scored as "successful". If a rat failed to grasp a piece of food, or grasped and dropped the food, the reach was scored as "unsuccessful".

After two weeks, the rats were retested and food was weighed before and after the 10 minute test. This was done in order to determine the amount of food eaten by each rat.

Place Navigation Task. This task is nearly identical to the Morris water task described in greater detail by Morris (1981) and is based upon a procedure devised by Sutherland, Whishaw, and Kolb (1983). The maze consisted of a circular pool (diameter, 150 cm; depth, 47.5 cm), the inside of which was painted white and filled to a height of 21.5 cm with approximately 18°C water in which 1.2 L of instant skim milk powder was dissolved. A clear Plexiglass platform (12.5 cm x 12.5 cm x 20.5 cm high) was present at a constant location inside the pool, 20 cm from the edge of the pool; its top surface was 1 cm below the surface of the opaque water, making the platform invisible to a viewer inside the pool.

A trial consisted of placing a rat by hand in the water, facing the wall of the pool, at one of four starting locations, north, south, east, or west, around the pool's perimeter. Within each block of four trials, each rat started at the four starting locations, but the sequence of locations was randomly selected. If on a particular trial a rat found the platform, it was permitted to remain on the platform for 10 seconds. A trial was terminated if a rat failed to find the platform after 60 seconds. At the end of a trial, the rat was returned to a holding cage, and approximately 5 min elapsed before beginning the next trial. The behavioral testing was conducted on five consecutive days, with each rat receiving four trials per day. On the final day a fifth trial was introduced in which the platform was moved and served as a probe trial to determine if the rats had learned the correct location of the platform.

Two measures of performance were taken on the place navigation task: escape latency (time required to find the platform) and heading error in the swim path. Escape latency was timed each day for every trial by the same

experimenter standing approximately 1 m from the pool's edge. On days 1 and 5, the swimming path for each rat on each trial was recorded on a map of the pool. The heading error in the swim path was determined by placing a 1 cm wide strip of cardboard across the map of the pool (diameter 4.75 cm) from the rat's starting position to the platform position. If the swim path remained within this 1 cm area, the rat was judged to be swimming directly toward the platform but, if the animal deviated from this path, the swim was scored as an error.

Landmark Navigation Task. The same 85 cm diameter pool was used as in the place navigation task. However, instead of keeping a constant location, the hidden platform was moved to a different quadrant of the tank for each block of trials. The location of the platform was marked by a black plastic rectangle (11 cm wide x 12 cm high) which hung on the pool wall directly behind the platform and was thus 20 cm away from the platform. The rats were trained as in the place navigation task except that the start location varied only among the three compass points distal to the platform. Thus, a trial block was made up of three, rather than four trials. One trial block was run per day for five consecutive days. The same two measures of performance were taken as on the place navigation task: escape latency and heading error.

Results

Behavioral Results

Forepaw Reaching. Normal rats learn to reach quickly and typically reach an asymptote of about 50-60% accuracy. Failed reaching attempts most commonly include the inadvertent grasping of several pieces of food, which are

usually dropped as the forepaw is withdrawn back to the body, as well as the occasional drop when food is grasped with both paws for eating. In this experiment, control rats made very precise reaching movements whereas rats with lesions made short, rapid, ineffective reaches. Rather than sitting on their haunches to eat, like control rats, most of the rats with frontal lesions lay prone and attempted to scoop pellets into their mouths.

In the first test, rats with bilateral frontal ablations made fewer successful reaches than control animals. Their poor performance reflects a larger number of errors similar to those made by normal animals, and may result from deficits in digit or paw use, limb trajectory, or posture (Whishaw, Pellis, & Gorny, 1992). In addition, in both the control and lesion groups, enriched-housed rats reached more successfully than laboratory-housed animals (Fig. 16). Two-factor ANOVA on Test 1 successful reaching scores showed significant main effects of lesion, $F(1,23) = 194.06, p < .0001$; and environment, $F(1,23) = 12.87, p < .0016$; but not of interaction, $F(1,23) = 2.52, p < .13$.

In the second test, 14 days later, control rats again made a higher percentage of successful reaches than animals with lesions (Fig. 16). Two-factor ANOVA on Test 2 successful reaching scores showed significant main effects of lesion, $F(1,23) = 234.62, p < .0001$; but not of environment, $F(1,23) = 5.01, p < .98$; nor of interaction, $F(1,23) = 0.26, p < .62$. The effects of enrichment observed in Test 1 declined as laboratory-housed animals in both the control and lesion groups increased their percentage of successful reaches in Test 2 to the extent that laboratory-housed controls performed as well as enriched-housed controls and the laboratory-housed frontal lesion group did not differ significantly from the enriched-housed frontal lesion group (Fig. 16).

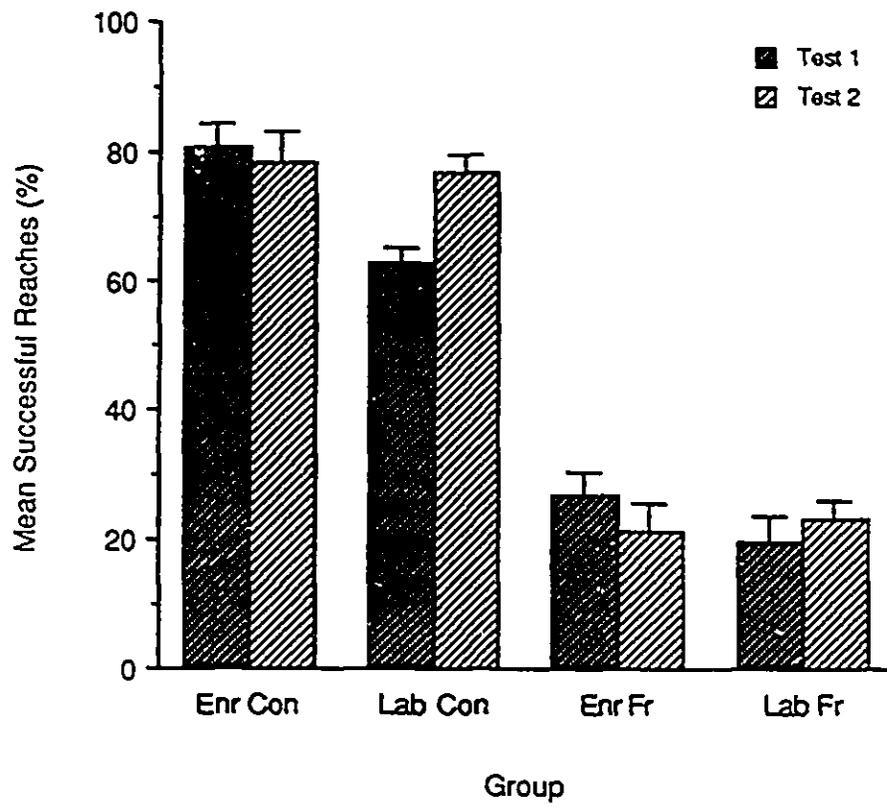


Figure 16. Summary of mean number of successful reaches (\pm SE) in a 10 minute period in Tests 1 and 2. **Enr Con**, enriched-housed control group; **Lab Con**, laboratory-housed control group; **Enr Fr**, enriched-housed frontal lesion group; **Lab Fr**, laboratory-housed frontal lesion group.

Three-factor repeated measures ANOVA was performed to look at the effects of lesion, environment and practice on successful reaches. It showed main effects of lesion, $F(1,20) = 343.42$, $p < .0001$; environment, $F(1,20) = 5.63$, $p < .0278$; and an interaction of environment and practice, $F(1,20) = 6.29$, $p < .0209$; but not interaction of lesion and environment, $F(1,20) = 2.13$, $p < .1596$.

The rats that were least successful in reaching for food made more reaches in each test. In the first test, rats with lesions reached more than control animals and laboratory-housed rats reached more than enriched-housed animals (Fig. 17). Two-factor ANOVA on Test 1 total reaching scores showed significant main effects of lesion, $F(1,23) = 63.45$, $p < .0001$; and environment, $F(1,23) = 4.57$, $p < .0434$; but not interaction, $F(1,23) = 0.03$, $p < .8681$. All the groups except the enriched-housed frontals made fewer total reaches in the second test than in the first test (Fig. 17). Two-factor ANOVA on Test 2 total reaching scores showed significant main effects of lesion, $F(1,23) = 73.24$, $p < .0001$; and interaction of lesion and environment, $F(1,23) = 5.00$, $p < .0353$; but not of environment, $F(1,23) = 2.38$, $p < .1366$. Three-factor repeated measures ANOVA was performed to look at the effects of lesion, environment and practice on total reaches. It showed main effects of lesion, $F(1,20) = 88.83$, $p < .0001$; interaction of lesion and practice, $F(1,20) = 7.9$, $p < .0108$; interaction of environment and practice, $F(1,20) = 7.64$, $p < .012$; and interaction of lesion, environment, and practice, $F(1,20) = 3.73$, $p < .0677$.

Curiously, even though control animals reached less and had a higher percentage of successful reaches than lesion animals, ANOVA revealed that the amount of food consumed by each of the four groups was not significantly different, $F(3,23) = 1.63$, $p < .21$.

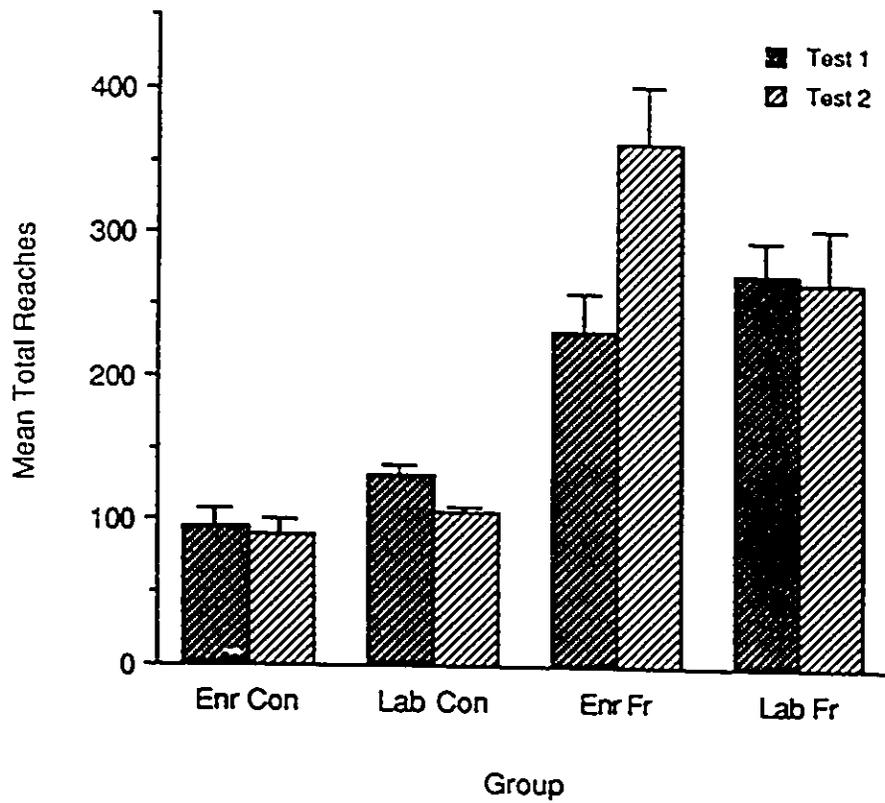


Figure 17. Summary of mean number of total reaches (\pm SE) in a 10 minute period in Tests 1 and 2. **Enr Con**, enriched-housed control group; **Lab Con**, laboratory-housed control group; **Enr Fr**, enriched-housed frontal lesion group; **Lab Fr**, laboratory-housed frontal lesion group.

Place Navigation Task. The behavior of rats in the place navigation task has been described in detail elsewhere (Morris, 1981). When initially placed in the pool, normal rats traverse a wide area, zig-zagging across the pool until they bump into the hidden platform. They then climb up on the platform and rear up a number of times. Performance of these rats improves rapidly on successive trials, and within about four trials they have reached asymptote, locating the platform from any start location within about 5 sec. When the position of the platform is moved in the probe trial, the rats initially swim around the region where the platform was previously located before exploring the rest of the tank.

The rats with frontal lesions initially used a different strategy to find the platform. Instead of zig-zagging across the pool, they swam along the wall until they bumped into the platform. Performance of rats with frontal lesions improved rapidly on successive trials and within four trial blocks they reached asymptote, locating the platform within about 7 sec from any start location. On the probe trial, rats with lesions performed in the same manner as control animals, that is, they searched for the platform in the previously correct location.

All groups improved with testing and learned the general location of the hidden platform (Fig. 18). Total latency scores showed that control rats performed better than rats with lesions and enriched-housed rats performed better than laboratory-housed animals (Fig. 19). Three-factor repeated measures ANOVA with lesion, practice, and environment as factors revealed a significant main effect of practice, $F(4,80) = 64.39$, $p < .0001$; but not of lesion, $F(1,20) = 1.52$, $p < .2324$; nor of environment, $F(1,20) = 0.92$, $p < .3482$.

Examination of the swim paths of the animals showed that both the

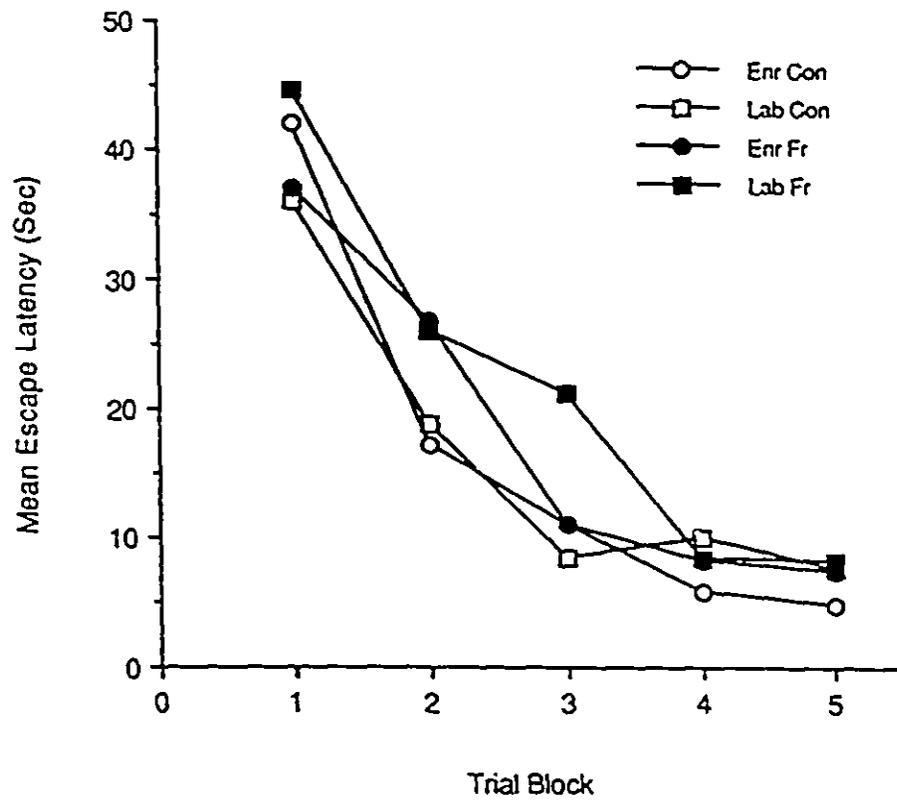


Figure 18. Summary of mean escape latency in the place navigation task for each of the 5 trial blocks. **Enr Con**, enriched-housed control group; **Lab Con**, laboratory-housed control group; **Enr Fr**, enriched-housed frontal lesion group; **Lab Fr**, laboratory-housed frontal lesion group.

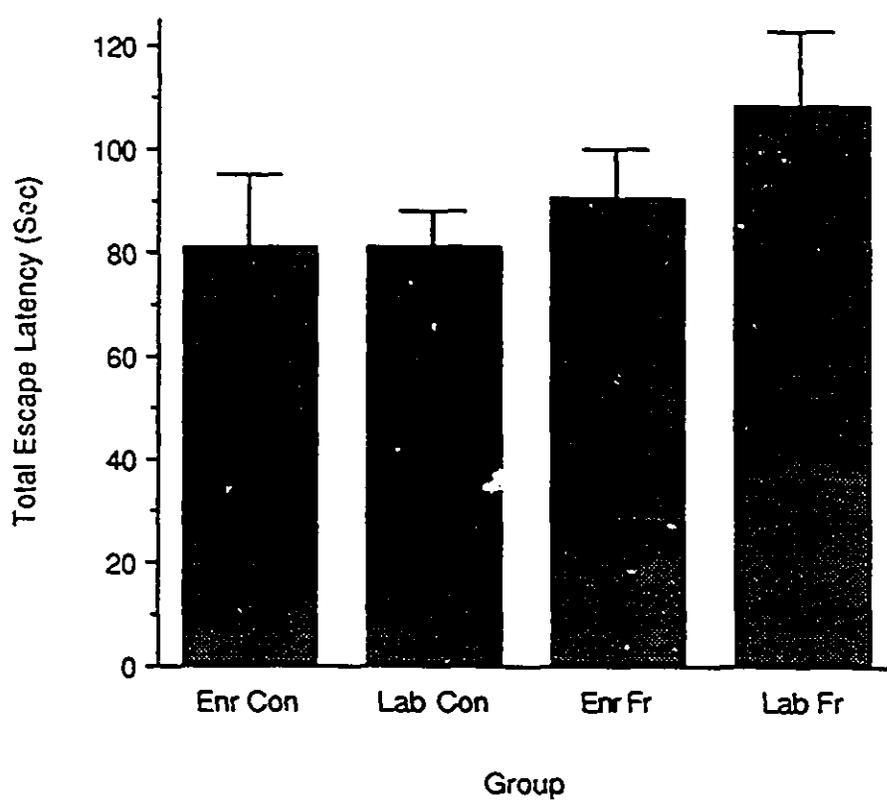


Figure 19. Summary of total escape latency (\pm SE) over 20 trials (5 trial blocks) in the place navigation task. **Enr Con**, enriched-housed control group; **Lab Con**, laboratory-housed control group; **Enr Fr**, enriched-housed frontal lesion group; **Lab Fr**, laboratory-housed frontal lesion group.

control and frontal rats learned to swim directly to the platform by the end of testing and there was no obvious effect of enrichment. Three-factor repeated measures ANOVA on the heading error of the first and last trial blocks revealed a significant effect of trial block, $F(1,24) = 40.51$, $p < .0001$; but not of lesion, environment, nor interaction.

Landmark Navigation Task. Even though all the groups learned to swim directly to the platform from any start location by the third trial block, rats with frontal lesions took longer to find the hidden platform (Fig 20). Total latency scores showed that control rats performed better than rats with lesions and enriched-housed rats performed better than laboratory-housed animals (Fig. 21). Three-factor repeated measures ANOVA with lesion, practice, and environment as factors was performed. It revealed a significant main effect of lesion, $F(1,20) = 4.02$, $p < .0587$; and practice, $F(4,80) = 3.1$, $p < .02$; but not of environment, $F(1,20) = 1.81$, $p < .1932$.

Examination of the swim paths of the animals showed that both the control and frontal rats learned to swim directly to the platform by the end of testing. Two-factor ANOVA on the heading error revealed a significant main effect of environment in trial block 5, $F(1,24) = 5.11$, $p < .0331$; and a significant interaction of lesion and environment in trial block 4, $F(1,24) = 5.35$, $p < .0296$.

Anatomical Results

Brain Weights. All the lesions removed the midline anterior cingulate and infralimbic cortex, and the medial region of Fr 1/2. The brain weights of the animals are summarized in Table 2 and show that (a) control rats had heavier brains than rats with lesions, and (b) enriched-housed rats in both groups had

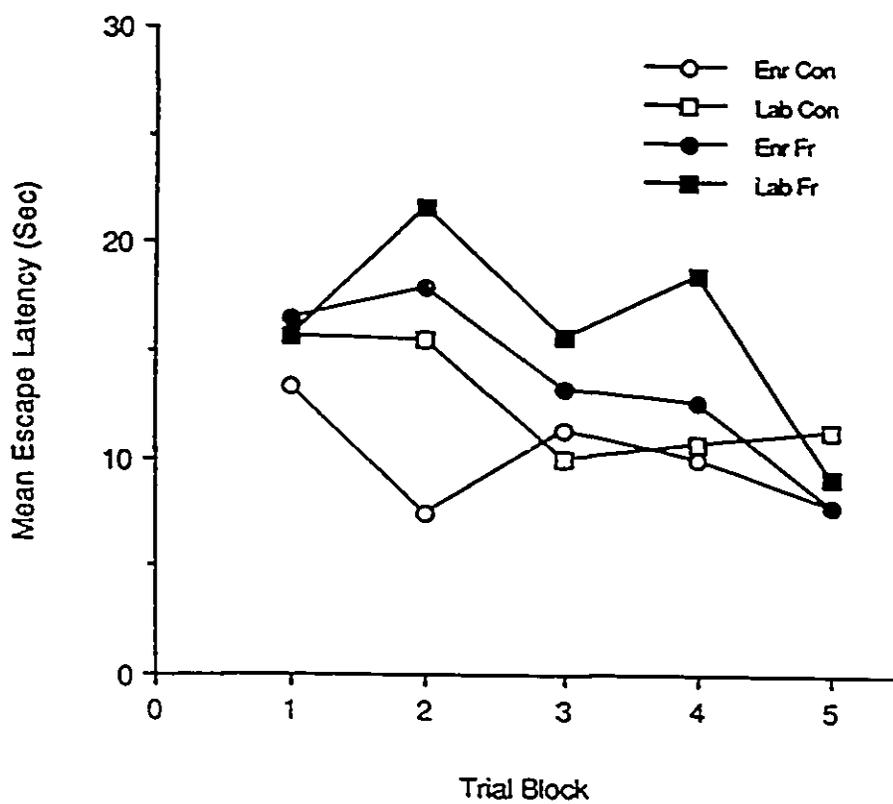


Figure 20. Summary of mean escape latency in the landmark navigation task for each of the 5 trial blocks. **Enr Con**, enriched-housed control group; **Lab Con**, laboratory-housed control group; **Enr Fr**, enriched-housed frontal lesion group; **Lab Fr**, laboratory-housed frontal lesion group.

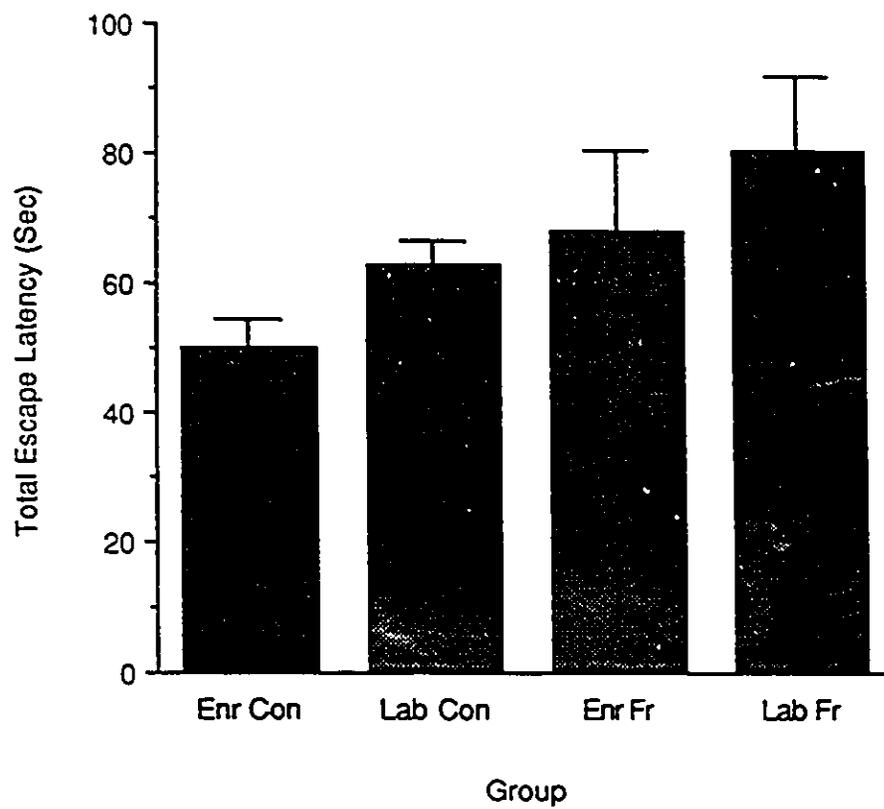


Figure 21. Summary of total escape latency (\pm SE) over 15 trials (5 trial blocks) in the landmark navigation task. **Enr Con**, enriched-housed control group; **Lab Con**, laboratory-housed control group; **Enr Fr**, enriched-housed frontal lesion group; **Lab Fr**, laboratory-housed frontal lesion group.

heavier brains than laboratory-housed rats. Two-factor ANOVA on brain weight showed a significant effect of lesion, $F(1,24) = 6.53$, $p < .0174$; but not of environment nor interaction. This finding may be misleading as one of the enriched control rats had an abnormally small brain (1.81 gm) as compared to the average weight of the other brains in that group (2.16 gm). When the data were analyzed without this rat, ANOVA on brain weight revealed significant main effects of lesion, $F(1,23) = 17.29$, $p < .0004$; and environment, $F(1,23) = 9.87$, $p < .0046$; but not of interaction, $F(1,23) = 0.43$, $p < .5195$.

The body weights of the animals are summarized in Table 3 and show that (a) the control rats weighed more than the rats with lesions, and (b) the enriched-housed rats in both groups weighed more than the laboratory-housed rats. Two-factor ANOVA on body weight showed significant main effects of lesion, $F(1,23) = 4.57$, $p < .0433$; and environment, $F(1,23) = 18.17$, $p < .0003$; but not of interaction, $F(1,23) = 3.26$, $p < .084$.

Cortical Thickness. Environmental enrichment appeared to have no effect on cortical thickness except at bregma -6.8 where laboratory-housed controls had thicker cortices at two places than the other three groups (Fig. 22). Frontal lesions appeared to have a non-specific effect throughout the cortex. At all three planes, rats with frontal lesions had thinner cortices than control rats (Fig. 22). Four-factor repeated measures ANOVA was performed to look at the effects of lesion, environment, anterior-posterior location (plane of measurement), and medial-lateral location (place of measurement) and revealed significant main effects of lesion, $F(1,60) = 21.78$, $p < .0001$; plane, $F(2,60) = 137.55$; $p < .0001$; place, $F(2,120) = 41.88$, $p < .0001$; and interaction of plane and place, $F(4,120) = 28.34$; $p < .0001$.

Table 2. Summary of brain weights

Group	Housing condition	
	Impoverished	Enriched
Control	2.04 ± 0.03	2.10 ± 0.06
Frontal	1.93 ± 0.03	2.01 ± 0.04

Numbers indicate means and SEs in grams.

Table 3. Summary of body weights

Group	Housing condition	
	Impoverished	Enriched
Control	416.3 ± 8.98	488.7 ± 19.19
Frontal	411.5 ± 10.70	477.0 ± 22.82

Numbers indicate means and SEs in grams.

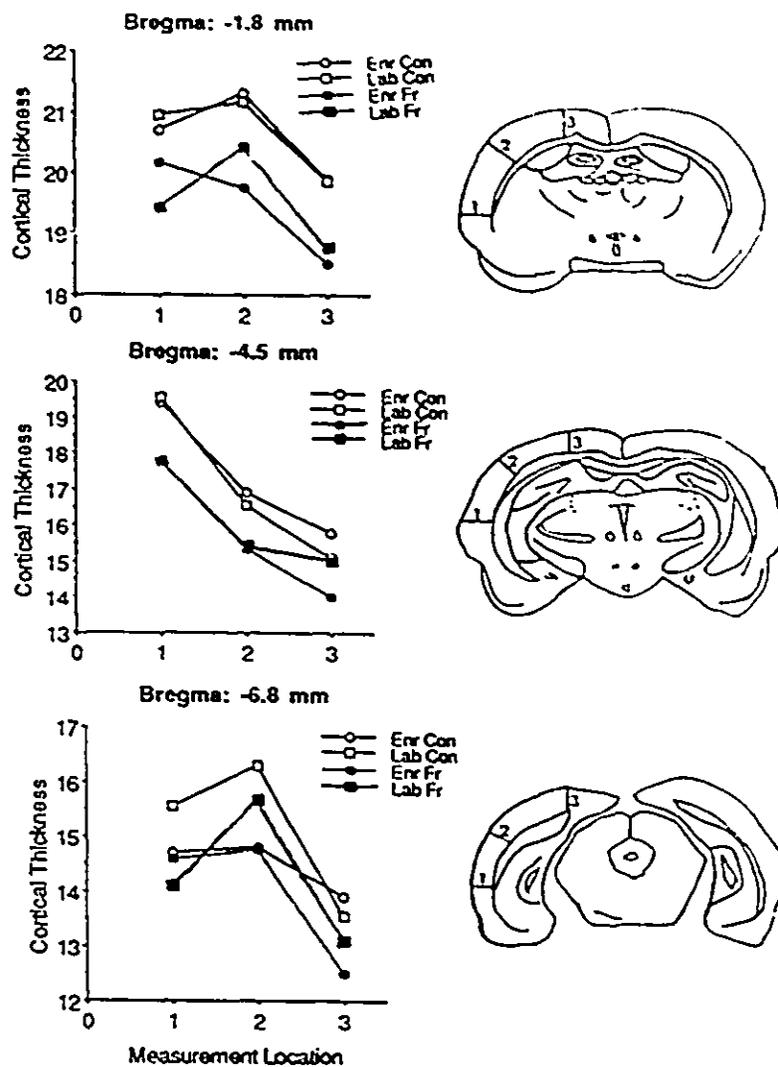


Figure 22. Summary of effects of enrichment on cortical thickness. The left panel illustrates the mean cortical thickness (13x) at each of 3 planes. Data points are the mean value for the right and left hemisphere measurements combined at each place of measurement at each plane of measurement. Right panel illustrates the points measured. Enr Con, enriched-housed control group; Lab Con, laboratory-housed control group; Enr Fr, enriched-housed frontal lesion group; Lab Fr, laboratory-housed frontal lesion group.

Discussion

Environmental enrichment prior to bilateral frontal ablation did not permanently attenuate post-lesion behavioral deficits. Similarly, enrichment did not enhance the performance of intact rats. Morphologically, pre-lesion enrichment increased brain weight in both intact and brain-injured animals but had no effect on cortical thickness.

Lesion Effects

Bilateral damage to the frontal cortex impaired performance in all tasks except the place navigation component of the water task. In the present experiment, when rats were tested in the water maze using five trial blocks of four trials each, rats with lesions performed as well as normal rats. Kolb and Gibb (1991a), using 10 trial blocks of eight trials each, found that rats with bilateral frontal lesions were severely impaired at this task and never became proficient. The difference in testing methods does not appear to be a determining factor in the results of this experiment, however, as rats with bilateral frontal damage were able to swim directly to the hidden platform within three trial blocks (like intact rats) and their escape latency was similar to that of intact rats. It is doubtful whether a longer testing period would have significantly improved the performance of either intact rats or rats with lesions, thus it appears that rats with frontal damage performed as well as intact rats and were not impaired at place navigation in the water task. It is possible that recovery of function in the present experiment may be task specific. If additional behavioral tests had been utilized, there may have been an environmental effect on recovery.

Kolb and Gibb (1991a) made large frontal lesions which included the ventral frontal cortex and the anterior part of the dorsal agranular insular cortex. In the present experiment, all of the midline anterior cingulate, the infralimbic cortex, and the medial region of Fr 1/2 were removed whereas the orbital region and the agranular insular cortex remained intact. One or both of these areas may be involved in mediating the spatial abilities required for normal performance in the water task.

An unexpected lesion effect was thinning of the entire cortex caudal to the lesions following damage to the frontal cortex. These findings are consistent with those of Kolb and Whishaw (1985) who found that removal of the medial frontal cortex in neonatal and adult rats resulted in significant thinning of the remaining cortex caudal to the lesion in both groups. On the other hand, Kolb and Gibb (1991b) found that frontal lesions induced increased dendritic branching in the parietal cortex of rats implying that the cortex may show a form of reactive synaptogenesis following injury.

Thinning of the cortex in areas caudal to the lesion could result from a disconnection of the remaining cortex from ascending or cortico-cortical projections. It is possible that this decrease in cortical depth may be due to alterations in catecholamine or other transmitter levels that are necessary for normal functioning of the brain. Because it has been suggested that noradrenaline is necessary for cortical plasticity (Sutherland, Kolb, Becker, & Whishaw, 1982), it seems likely that interruption of noradrenergic input to the residual cortex could affect brain morphology and function. Since noradrenergic fibers course through the anterior cortex en route to the posterior cortex, this could explain why the major cortical changes appear caudal to the

lesion sites. The occurrence of abnormal cortical morphology following frontal lesions is significant for it may account, at least in part, for the occurrence of behavioral deficits that are not associated with the location of the lesion.

Enrichment Effects

Pre-lesion environmental enrichment attenuated behavioral deficits in rats with frontal lesions in the reaching task. In the initial test, rats housed in an enriched environment prior to surgery were more successful in obtaining food than were animals housed in standard laboratory cages but the effects of environmental manipulation disappeared by the time the animals were re-tested, two weeks later. In the second test, the enriched-housed rats were not as successful as they were in the first test and the laboratory-housed rats (both control and frontal lesion groups) were as successful as the enriched-housed rats at securing food. There are two possible explanations for this change in performance: (a) The enrichment effect disappeared because the enriched rats were housed in standard laboratory cages following surgery, and (b) there was a training effect in the laboratory-housed rats because they were subjected to other behavioral tests during the period of time between the two reaching tests.

Environmental manipulation had no effect on performance in the Morris water task: Laboratory-housed rats performed as well as enriched-housed rats. These findings are consistent with those of Kolb and Gibb (1991a) who found no enrichment effect in the water task when rats received postoperative environmental enrichment following frontal cortex lesions. Because environmental manipulation in the present experiment had a transient effect on the reaching task but not on the water task, it would be reasonable to conclude that enrichment-induced recovery from frontal cortex lesions is task-specific.

The rats housed in an enriched environment prior to frontal injury weighed more than laboratory-housed animals and they also had heavier brains. Curiously, even though the enriched-housed rats had heavier brains, the cortices were not significantly thicker than those of rats housed in standard laboratory cages. These results can be accounted for in three ways: (a) The increase in cortical thickness induced by pre-lesion enrichment did not persist resulting in thinning of the cortex during post-lesion standard laboratory housing, (b) a training effect in the laboratory-housed rats during post-lesion behavioral testing induced an increase in cortical thickness, or (c) the heavier brains of the enriched rats were due solely to their larger body size.

Putative bFGF Effects

The enhanced astrocytic expression of endogenous bFGF induced by environmental enrichment (observed in Experiment 3) did not permanently attenuate post-lesion behavioral deficits in the present experiment. However, there was a transient beneficial effect on reaching performance that disappeared after two weeks. In addition, enhancing injury-induced bFGF expression (which may have prevented neuritic degeneration in Experiment 2) did not result in an increase in cortical thickness in this experiment.

There are three possible explanations that would account for the lack of behavioral and morphological response to pre-lesion environmental enrichment. First, there may be an optimal level of endogenous bFGF that is necessary to attenuate post-lesion behavioral deficits and to sustain injury-induced neuritic outgrowth. Experiment 2 demonstrated that blocking the injury-induced astrocytic expression of bFGF prevents post-lesion recovery of behavioral function and provokes neuritic degeneration, whereas normal

expression of endogenous bFGF following cortical injury results in performance levels and neuritic outgrowth similar to those of intact rats. On the other hand, increased astrocytic expression of endogenous bFGF induced by environmental enrichment (observed in Experiment 3) did not enhance either post-lesion performance or injury-induced neuritic outgrowth (i.e., cortical thickness) in the present experiment. These results suggest that (a) there is a limit to the amount of endogenous bFGF that is beneficial following brain injury, (b) normal injury-induced expression of endogenous bFGF is optimal, and (c) enhancing the expression of endogenous bFGF with pre-lesion environmental enrichment alone is ineffective.

Second, continuously enhanced levels of bFGF in the injured brain may be necessary to reduce post-lesion behavioral deficits and to sustain neuritic outgrowth. The enhanced levels of endogenous bFGF induced by pre-lesion environmental enrichment may have declined when rats were transferred from the enriched environment to standard laboratory cages. This decline in bFGF levels may be responsible for an increase in behavioral deficits and an escalation of neuritic degeneration resulting in loss of the increased cortical thickness achieved during enrichment. Thus, post-lesion environmental enrichment may also be necessary to sustain the morphological and behavioral effects.

Third, increased post-lesion expression of endogenous bFGF induced by pre-lesion environmental enrichment may not be sufficient to enhance neuritic outgrowth and to attenuate injury-induced behavioral deficits. In addition, increased bFGF levels may not be present early enough in the regeneration sequence. In order to produce beneficial results, it may be

necessary to supplement injury-induced endogenous bFGF with exogenous bFGF. Invasive procedures that introduce exogenous bFGF into injured rat brain enhance neuronal survival and promote neuritic outgrowth (Cummings et al, 1992; Gomez-Pinilla et al, 1992; Miyamoto et al, 1993). These studies suggest that introducing sufficient amounts of bFGF into the injured brain at appropriate times in the regeneration sequence enhances the morphological injury response and may subsequently attenuate behavioral deficits.

GENERAL DISCUSSION

Basic FGF (also referred to as FGF-2) is one member of a family of seven homologous CNS proteins (Vlodavsky, Bar Shavit, Ishai Michaeli, Bashkin, & Fuks, 1991). In the intact adult rat brain bFGF is found in neurons and glia, in the vascular basement membrane of blood vessels, and in the ependymal cells lining the ventricles (Cuevas, Gimenez-Gallego, Martinez-Murillo, & Carceller, 1991). Glia and neurons synthesize bFGF (Emoto et al., 1989) and its receptors (Wanaka, Johnson, & Milbrandt, 1990) throughout the brain but particularly high levels of bFGF expression are seen in several neural structures such as field CA2 of the hippocampus, the amygdala, the cingulate cortex, and the piriform cortex.

In the developing rat brain, bFGF may be important for the maturation of both acetylcholine- and dopamine-containing neurons (Gonzalez, Buscaglia, Ong, & Baird, 1990). *In vitro*, bFGF promotes survival and enhances neuritic outgrowth in a wide range of fetal cells including cortical, hippocampal, striatal, septal, hypothalamic, mesencephalic, and ciliary ganglionic neurons (Baird & Bohlen, 1990).

As well as being involved in the development and maintenance of the rat brain, bFGF appears to play an important role in the trophic response to brain injury. The primary purpose of the research undertaken in this thesis was to examine the effects of endogenous bFGF on brain plasticity and recovery of behavioral function following cortical injury in adult rats. The first experiment was performed to investigate the time course of astrocytic expression of bFGF following motor cortex injury. Three subsequent experiments examined the

effects of injury-induced bFGF on neuronal morphology, cortical morphology, and post-lesion behavioral deficits. Collectively, these experiments suggest that in response to cortical injury, endogenous bFGF is expressed by astrocytes surrounding the lesion site, whereupon it prevents neuritic degeneration and ultimately contributes to recovery of behavioral function.

Other studies have provided evidence that bFGF promotes neuronal survival and neurite extension *in vitro* (Grothe et al., 1989; Walicke, 1988) and *in vivo* (Anderson et al., 1988; Otto et al., 1989; Sievers et al., 1987) but no studies have been conducted that might establish a role for bFGF in recovery of behavioral function. The results of the present research indicate that injury-induced bFGF promotes recovery of function that is associated with dendritic branching frequency and dendritic spine density similar to that observed in rats with intact brains. Housing rats in an enriched environment enhances astrocytic expression of bFGF but pre-lesion environmental enrichment alone does not reduce post-lesion behavioral deficits nor increase cortical thickness (relative to laboratory-housed rats). These results suggest that there may be a limit to the amount of bFGF that is beneficial following cortical injury. Thus, recovery of function and prevention of neuritic degeneration may not depend on absolute amounts of bFGF available immediately following injury, but may rely on bFGF as a single component in a multifactorial cascade of regenerative events.

There is considerable evidence that bFGF participates in the trophic response to injury, however, it is apparent that many other trophic factors contribute to the regenerative response following brain injury. Multiple growth factors are locally released after brain injury and interact to control regenerative cellular changes. For example, an interaction between bFGF and nerve growth

factor (NGF) demonstrates that growth factors may coordinate their actions following brain injury. Basic FGF-responsive neurons express NGF receptors (Gomez-Pinilla et al., 1992) implying that these cells may be responsive to NGF. Astrocytes synthesize NGF *in vitro* (Furukawa, Furukawa, Satoyoshi, & Hayashi, 1986) and bFGF enhances NGF concentration in astrocyte cell cultures (Yoshida & Gage, 1991) and *in vivo* (Yoshida & Gage, 1992). Thus, the bFGF-stimulated expression of astrocytic NGF suggests that both bFGF and NGF would be made available to damaged neurons and the interaction of these two growth factors could facilitate neuronal survival and regeneration.

The results of the research undertaken in this thesis suggest that bFGF is a single component in an injury response that is beneficial to neuronal survival and regeneration but the question arises whether this cellular regenerative response contributes to functional reconnection of damaged neural pathways and subsequently to recovery of behavioral function. General cellular events following injury to the adult rat brain seem to be directed to survival but not to restoration of functional circuitry. Primary neuronal death occurs immediately following cortical injury and secondary neuronal death takes place about four days later. During the first few days, repaired blood vessels restore circulation and macrophage invasion begins. Two days after brain injury, glial cell proliferation begins and after five days, a vascularized glial scar can be observed. Reactive sprouts (from undamaged neurons) innervate deafferented cells beginning four days after the lesion whereas regenerative sprouts (from damaged neurons) do not begin to form until 8 to 10 days after the lesion (Nieto-Sampedro & Cotman, 1985).

At this point, two potentially deleterious effects of the injury response

become apparent. First, since secondary neuronal death is essentially complete four days after brain injury occurs and bFGF peaks seven days after the injury, trophic activity is not sufficiently rapid to provide adequate support to a large proportion of injured neurons. Second, regenerating axons are prevented from reaching their normal target by the combined effects of the newly formed glial scar and by the rapid and vigorous reinnervation of their targets by the reactive sprouts of undamaged axons. Regenerative sprouts either degenerate or form inappropriate synapses proximal to their site of origin. Obviously, some kind of intervention is necessary to enhance neuronal survival and to encourage more rapid regeneration of damaged neurons.

It is feasible that exogenously supplied bFGF (and/or other trophic factors), if supplied immediately after injury in sufficient amounts, could enhance the survival and regeneration of neurons affected by injury and could promote functional reconnection of injured neural pathways. A major obstacle to supplying exogenous bFGF is the blood-brain barrier. Systemically circulating proteins do not readily cross the blood-brain barrier therefore invasive techniques have been adopted as the main method for the delivery of neurotrophic factors to the injured brain. Single or repeated injections and osmotic minipumps (Morse et al., 1993; Widmer, Knusel, & Hefti, 1993) have been used in laboratory animals to infuse neurotrophins directly into the brain.

In an effort to develop non-invasive techniques, investigators have demonstrated that systemically administered neurotrophins can cross the blood-brain barrier in the rat brain when they are conjugated to a carrier protein. For example, Friden et al. (1993) used the transferrin receptor, a transporter of iron across capillary cells, to transport NGF through the blood-brain barrier.

Regardless of whether invasive or non-invasive methods are used, increasing the levels of neurotrophic factors in the brain has several limitations. First, over-expression or increased release of bFGF may be involved in the formation of astrocytomas (Eckenstein et al., 1994). Second, the infusion of bFGF must occur at a specific time in the regeneration sequence. For example, topical application of bFGF to tissue surrounding somatosensory cortex lesions in rats prevents retrograde neuronal degeneration only when applied immediately after injury (Kohmura et al., 1994). Third, injured neurons are temporarily rescued when neurotrophic factors are infused into the lesion site but most of the rescued neurons die soon after infusion is discontinued (Gage, Armstrong, Williams, & Varon, 1988; Mansour-Robaey, Clarke, Wang, Bray, & Aguayo, 1994). Thus, in order for increased levels of neurotrophic factors to be effective, administration of appropriate amounts of exogenous neurotrophins must be initiated at a specific time in the regeneration sequence, and may have to be sustained indefinitely.

The results of this research have implications for humans with brain damage. Pre-lesion environmental enrichment, or in the case of humans, physical and cognitive stimulation, is often considered to provide "protection" from the effects of brain trauma. The results of this study indicate that "protection" is not effective in enhancing recovery from brain damage. Post-lesion environmental enrichment, or stimulation, is described as post-traumatic "therapy" (Stein, Finger, & Hart, 1983). In fact, the usual course of action following brain injury in humans is to initiate a course of physical therapy. Therapy consists of supervised practice of impaired behaviors and thus, concentrates on treating the effects of brain damage, not the actual cause of the

disabilities, which is the injured brain. It is feasible that post-traumatic "therapy" may eventually be complemented by administration of bFGF (and/or other trophic factors) to the injured brain.

Administration of neurotrophic factors may play an important therapeutic role in preventing neuronal degeneration and promoting neuronal regeneration following brain injury. Nevertheless, improvements in the *in vivo* delivery of neurotrophic factors and a better understanding of the actions of these factors in the injured brain are essential before effective therapies can be developed for the treatment of brain injury.

REFERENCES

- Anderson, K. J., Dam, D., Lee, S., & Cotman, C. W. (1988). Basic fibroblast growth factor prevents death of lesioned cholinergic neurons in vivo. Nature, 332, 360-361.
- Baird, A., & Bohlen, P. (1990). Fibroblast growth factors. In M. B. Sporn, & A. B. Roberts (Eds.), Peptide growth factors and their receptors. I. Handbook of experimental pharmacology: Vol. 95 (pp. 369-418). Berlin: Springer Verlag.
- Cohen, S. (1960). Purification of a nerve growth promoting protein from the mouse salivary gland and its neurotoxic antiserum. Proceedings of the National Academy of Sciences, USA, 46, 302-311.
- Coleman, P. D., & Flood, D. G. (1987). Neuron numbers and dendritic extent in normal aging and Alzheimer's disease. Neurobiology of Aging, 8(6), 521-545.
- Coleman, P. D., & Flood, D. G. (1988). Is dendritic proliferation of surviving neurons a compensatory response to loss of neighbors in the aging brain? In S. Finger, T. E. LeVere, C. R. Almlı, & D. G. Stein (Eds.), Brain injury and recovery: Theoretical and controversial issues (pp. 235-247). New York: Plenum Press.
- Coleman, P. D., & Riesen, A. H. (1968). Environmental effects on cortical dendritic fields. I. Rearing in the dark. Journal of Anatomy, 102, 363-374.
- Connor, J. R., & Diamond, M. C. (1982). A comparison of dendritic spine number and type on pyramidal neurons of the visual cortex of old adult rats from social or isolated environments. The Journal of Comparative Neurology, 210, 99-106.
- Cooper, R. M., Blochert, K. P., Gillespie, L. A., & Miller, L. C. (1972). Translucent occluders and lesions of posterior cortex in the rat. Physiology and Behavior, 8, 693-697.
- Cotman, C. W., Cummings, B. J., & Pike, C. J. (1993). Molecular cascades in adaptive versus pathological plasticity. In A. Gorio (Ed.), Neurodegeneration (pp. 217-240). New York: Raven Press.

- Cotman, C. W., Matthews, D. A., Taylor, D., & Lynch, G. (1973). Synaptic rearrangement in the dentate gyrus: Histochemical evidence of adjustments after lesions in immature and adult rats. Proceedings of the National Academy of Sciences, USA, 70, 3473-3477.
- Cotman, C. W., & Nadler, J. V. (1978). Reactive synaptogenesis in the hippocampus. In C. W. Cotman (Ed.), Neuronal plasticity (pp. 227-271). New York: Raven Press.
- Cotman, C. W., Nieto-Sampedro, M., & Harris, E. W. (1981). Synapse replacement in the nervous system of adult vertebrates. Physiological Reviews, 61, 684-784.
- Cuevas, P., Gimenez-Gallego, G., Martinez-Murillo, R., & Carceller, F. (1991). Immunohistochemical localization of basic fibroblast growth factor in ependymal cells of the rat lateral and third ventricles. Acta Anatomica, 141, 307-310.
- Cummings, B. J., Yee, G. J., & Cotman, C. W. (1992). bFGF promotes the survival of entorhinal layer II neurons after perforant path axotomy. Brain Research, 591(2), 271-276.
- Dalrymple-Alford, J. C., & Kelche, C. (1985). Behavioral effects of preoperative and postoperative differential housing in rats with brain lesions: A review. In B. Will, P. Schmitt, & J. C. Dalrymple-Alford (Eds.), Brain plasticity, learning, and memory. Advances in behavioral biology (pp. 441-458). New York: Plenum Press.
- Dalrymple-Alford, J. C., & Kelche, C. (1987). Behavioral effects of differential postoperative housing after septal lesions made in weanling rats. Psychobiology, 3, 255-260.
- Date, I., Yoshimoto, Y., Imaoka, T., Yasuyuki, M., Gohda, Y., Furuta, T., Asari, S., & Ohmoto, T. (1993). Enhanced recovery of the nigrostriatal dopaminergic system in MPTP-treated mice following intrastriatal injection of basic fibroblast growth factor in relation to aging. Brain Research, 621, 150-154.
- deLacoste, M. C., & Horvath, D. S. (1985). Sex differences in the development of morphological asymmetries in human fetuses. American Journal of Physical Anthropology, 66, 163.

- Diamond, M. C. (1967). Extensive cortical depth measurements and neuron size increase in the cortex of environmentally enriched rats. The Journal of Comparative Neurology, 131, 357-364.
- Diamond, M., Dowling, G., & Johnson, R. (1981). Morphologic cerebral cortex asymmetry in male and female rats. Experimental Neurology, 71, 261-268.
- Diamond, M. C., Ingham, C. A., Johnson, R. E., Bennett, E. L., & Rosenzweig, M. R. (1976). Effects of environment on morphology of rat cerebral cortex and hippocampus. Journal of Neurobiology, 7, 75-85.
- Diamond, M., Law, F., Rhodes, H., Lindner, B., Rosenzweig, M., Krech, D., & Bennett, E. L. (1966). Increases in cortical depth and glia numbers in rats subjected to enriched environment. The Journal of Comparative Neurology, 128, 117-125.
- Diamond, M. C., Rosenzweig, M. R., Bennett, E. L., Lindner, B., & Lyon, L. (1972). Effects of environmental enrichment and impoverishment on rat cerebral cortex. Journal of Neurobiology, 3, 47-64.
- Donovick, P. J., Burrig, R. C., & Bentsen, E. D. (1975). Presurgical dietary history and the behavior of control and septal lesioned rats. Developmental Psychobiology, 8, 13-26.
- Donovick, P. J., Burrig, R. C., & Swidler, M. A. (1973). Presurgical rearing environment alters exploration, fluid consumption and learning of septal lesioned and control rats. Physiology and Behavior, 11, 543-553.
- Eccleston, P. A., & Silberberg, D. H. (1985). Fibroblast growth factor is a mitogen for oligodendrocytes in vitro. Developmental Brain Research, 21, 315-318.
- Eckenstein, F. P., Kuzis, K., Nishi, R., Woodward, W. R., Meshul, C., Sherman, L., & Ciment, G. (1994). Cellular distribution, subcellular localization and possible functions of basic and acidic fibroblast growth factors. Biochemical Pharmacology, 47(1), 103-110.
- Eidelberg, E., Stein, D. G. (1974). Functional recovery after lesions of the nervous system. Neurosciences Research Program Bulletin, 12, 191-303.

- Emoto, N., Gonzalez, A. M., Walicke, P. A., Wada, E., Simmons, D. M., Shimasaki, S., & Baird, A. (1989). Identification of specific loci of basic fibroblast growth factor synthesis in the rat brain. Growth Factors, *2*, 21-29.
- Finklestein, S. P., Apostolides, P. J., Caday, C. G., Prosser, J., Philips, M. F., & Klagsbrun, M. (1988). Increased basic fibroblast growth factor (bFGF) immunoreactivity at the site of focal brain wounds. Brain Research, *460*, 253-259.
- Fischer, W., Victorin, K., Bjorklund, A., Williams, L. R., & Gage, F. H. (1987). Amelioration of cholinergic neurotrophs and spatial memory impairment in aged rats by NGF. Nature, *329*, 65-68.
- Friden, P. M., Walus, L. R., Watson, P., Doctrow, S. R., Kozarich, J. W., Backman, C., Bergman, H., Hoffer, B., Bloom, F., Granholm, A-C. (1993). Blood-brain barrier penetration and in vivo activity of an NGF conjugate. Science, *259*, 373-377.
- Furukawa, S., Furukawa, Y., Satoyoshi, E., & Hayashi, K. (1986). Synthesis and secretion of nerve growth factor by mouse astroglial cells in culture. Biochemistry and Biophysics Research Communications, *136*, 57-63.
- Gage, F. H., Armstrong, D. M., Williams, L. R., & Varon, S. (1988). Morphologic responses of axotomized septal neurons to nerve growth factor. The Journal of Comparative Neurology, *269*, 147-155.
- Geschwind, N. (1965). Disconnection syndromes in animals and man. Brain, *88*, 237-294, 585-644.
- Geschwind, N., & Galaburda, A. M. (1987). Cerebral lateralization: Biological mechanisms, associations and pathology. Cambridge, MA: MIT Press.
- Glaser, E. M., & van der Loos, H. (1981). Analysis of thick brain sections by obverse-reverse computer microscopy: Application of a new, high clarity Golgi-Nissl stain. Journal of Neuroscience Methods, *4*, 117-125.
- Glassman, R. B., & Smith, A. (1988). Neural spare capacity and the concept of diaschisis: Functional and evolutionary models. In S. Finger, T. E. LeVere, C. R. Almlie, & D. G. Stein (Eds.), Brain injury and recovery: Controversial and theoretical issues (pp.45-69). New York: Plenum.

- 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 and Physiological Psychology, *82*, 175-181.
- Goldman-Rakic, P., & Brown, R. M. (1981). Regional changes of monoamines in cerebral cortex and subcortical structures of aging rhesus monkeys. Neuroscience, *6*, 177-187.
- Goldstein, M. (1990). Traumatic brain injury: A silent epidemic. Annals of Neurology, *27*, 327.
- Gomez-Pinilla, F., Lee, J. W-K., & Cotman, C. W. (1992). Basic FGF in adult rat brain: Cellular distribution and response to entorhinal lesion and fimbria-fornix transection. The Journal of Neuroscience, *12*(1), 345-355.
- Gonzalez, A. M., Buscaglia, M., Ong, M., & Baird, A. (1990). Immunohistochemical localisation of basic FGF in tissues of the 18 day foetal rat. Journal of Cell Biology, *110*, 347-358.
- Greenough, W. T. (1976). Enduring brain effects of differential experience and training. In M. R. Rosenzweig, & E. L. Bennett (Eds.), Neural mechanisms of learning and memory (pp. 255-278). Massachusetts: MIT Press.
- Greenough, W. T., & Volkmar, R. F. (1973). Pattern of dendritic branching in occipital cortex of rats reared in complex environments. Experimental Neurology, *40*, 491-504.
- Greenough, W. T., Volkmar, R. F., & Juraska, J. M. (1973). Effects of rearing complexity on dendritic branching in frontolateral and temporal cortex of the rat. Experimental Neurology, *41*, 371-378.
- Grothe, C., Otto, D., & Unsicker, K. (1989). Basic fibroblast growth factor promotes in vitro survival and cholinergic development of septal neurons: Comparisons with the effects of nerve growth factor. Neuroscience, *31*, 649-661.
- Hicks, S., & D'Amato, C. J. (1970). Motor-sensory and visual behavior after hemispherectomy in newborn and mature rats. Experimental Neurology, *29*, 416-438.
- Hinds, J. W., & McNelly, N. A. (1977). Aging of the rat olfactory bulb: Growth and atrophy of constituent layers and changes in size and number of mitral cells. The Journal of Comparative Neurology, *171*, 345-368.

- Hughes, K. R. (1965). Dorsal and ventral hippocampus lesions and maze learning: Influence of preoperative environment. Canadian Journal of Psychology, 19, 325-332.
- Irle, E. (1987) Lesion size and recovery of function: Some new perspectives. Brain Research Review, 12, 307-320.
- 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.
- Kasamatsu, T., & Pettigrew, J. D. (1976). Depletion of brain catecholamines: Failure of ocular dominance shift after monocular occlusion in kittens. Science, 194, 206-209.
- Kasamatsu, T., & Pettigrew, J. D. (1979). Preservation of binocularity after monocular deprivation in the striate cortex of kittens treated with 6-hydroxydopamine. The Journal of Comparative Neurology, 185, 139-162.
- Kelche, C., Dalrymple-Alford, J., & Will, B. (1987). Effects of postoperative environment on recovery of function after fimbria-fornix transection in the rat. Physiology and Behavior, 40, 731-736.
- Kennard, M. A. (1936). Age and other factors in motor recovery from precentral lesions in monkeys. American Journal of Physiology, 115, 138-146.
- Kennard, M. A. (1938). Reorganization of motor function in the cerebral cortex of monkeys deprived of motor and premotor areas in infancy. Journal of Neurophysiology, 1, 477-496.
- Kimura, D. (1983). Sex differences in cerebral organization for speech and praxic functions. Canadian Journal of Psychology, 37, 19-35.
- Kohmura, E., Yuguchi, T., Yamada, K., Sakaguchi, T., & Hayakawa, T. (1994). Recombinant basic fibroblast growth factor spares thalamic neurons from retrograde degeneration after ablation of the somatosensory cortex in rats. Restorative Neurology and Neuroscience, 6, 309-316.
- Kolb, B. (1984). Functions of frontal cortex of the rat: A comparative review. Brain Research Review, 8, 65-98.

- Kolb, B. (1987). Recovery from early cortical damage in rats. I. Differential behavioral and anatomical effects of frontal lesions at different ages of neural maturation. Behavioral Brain Research, *25*, 205-220.
- Kolb, B. (1989). Brain development, plasticity, and behavior. American Psychologist, *44*(9), 1203-1212.
- Kolb, B. (1992). Mechanisms underlying recovery from cortical injury: Reflections on progress and directions for the future. In F. D. Rose, & D. A. Johnson (Eds.), Recovery from brain damage (pp.169-186). New York: Plenum Press.
- 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. (1991a). Environmental enrichment and cortical injury: Behavioral and anatomical consequences of frontal cortex lesions. Cerebral Cortex, *1*, 189-198.
- Kolb, B., & Gibb, R. (1991b). Sprouting of function after neonatal frontal lesions correlates with increased cortical dendritic branching: A possible mechanism for the Kennard effect. Behavioral Brain Research, *43*, 51-56.
- Kolb, B., & Gibb, R. (1993). Possible anatomical basis of recovery of function after neonatal frontal lesions in rats. Behavioral Neuroscience, *107*(5), 799-811.
- Kolb, B., Gibb, R., & van der Kooy, D. (1992). Cortical and striatal structure and connectivity are altered by neonatal hemidecortication in rats. The Journal of Comparative Neurology, *322*, 311-324.
- 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., Ladowski, R., Gibb, R., & Gorny, G. (1995). Does dendritic growth underly recovery from neonatal occipital lesions in rats? Behavioral Brain Research.

- Kolb, B., & McLimans, J. (1986). A process for cryostat sectioning of Golgi-Cox tissue. Stain Technology, *61*, 379-380.
- Kolb, B., & Sutherland, R. J. (1986). A critical period for noradrenergic modulation of sparing from neocortical parietal cortex damage in the rat. Society of Neurosciences Abstracts, *12*, 322.
- Kolb, B., & Sutherland, R. J. (1992). Noradrenaline depletion blocks behavioral sparing and alters cortical morphogenesis after neonatal frontal cortex damage in rats. The Journal of Neuroscience, *12*(6), 2321-2330.
- Kolb, B., & Tomie, J. (1988). Recovery from early cortical damage in rats. IV. Effects of hemidecortication at 1, 5, or 10 days of age on cerebral anatomy and behavior. Behavioral Brain Research, *28*, 259-284.
- Kolb, B., & Whishaw, I. Q. (1981a). Decortication in rats in infancy or adulthood produced comparable functional losses on learned and species-typical behaviors. Journal of Comparative and Physiological Psychology, *95*, 468-483.
- Kolb, B., & Whishaw, I. Q. (1981b). 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. (1983). Dissociation of the contributions of the prefrontal, motor, and parietal cortex to the control of movement in the rat: An experimental review. Canadian Journal of Psychology, *37*, 211-232.
- Kolb, B., & Whishaw, I. Q. (1985). Earlier is not always better: Behavioral dysfunction and abnormal cerebral morphogenesis following neonatal cortical lesions in the rat. Behavioral Brain Research, *17*, 25-43.
- Kolb, B., & Whishaw, I. Q. (1989). Plasticity in the neocortex: Mechanisms underlying recovery from early brain damage. Progress in Neurobiology, *32*, 235-276.
- Kolb, B., & Whishaw, I. Q. (1990). Fundamentals of human neuropsychology. New York: W. H. Freeman and Company.

- Kolb, B., & Whishaw, I. Q. (1991). Mechanisms underlying behavioral sparing after neonatal retrosplenial cingulate lesions in rats: Spatial navigation, cortical architecture, and electroencephalographic activity. Brain Dysfunction, *4*, 75-92.
- Korsching, S. (1993). The neurotrophic factor concept: A reexamination. The Journal of Neuroscience, *13*(7), 2739-2748.
- Lashley, K. S. (1929). Brain mechanisms and intelligence. Chicago: University of Chicago.
- Lee, K., Stanford, E., Cotman, C. W., & Lynch, G. S. (1977). Ultrastructural evidence for bouton sprouting in the adult mammalian brain. Experimental Brain Research, *29*, 475-485.
- Lennenberg, E. (1967). Biological foundations of language. New York: John Wiley.
- Levi-Montalcini, R., & Booker, B. (1960). Destruction of the sympathetic ganglia in mammals by an antiserum to the nerve growth promoting factor. Proceedings of the National Academy of Sciences, USA, *42*, 384-391.
- Levi-Montalcini, R., & Cohen, S. (1960). Effects of the extract of the mouse submaxillary salivary glands on the sympathetic system of animals. Annals of the New York Academy of Sciences, *85*, 324-341.
- Liu, Z., D'Amore, P., Mikati, M., Gatt, A., & Holmes, G. (1993). Neuroprotective effect of chronic infusion of basic fibroblast growth factor on seizure-associated hippocampal damage. Brain Research, *626*, 335-338.
- Logan, A., & Berry, M. (1993). Transforming growth factor-B1 and basic fibroblast growth factor in the injured CNS. Trends in Pharmacological Sciences, *14*, 337-342.
- Logan, A., Frautschy, S. A., Gonzalez, A-M., & Baird, A. (1992). A time course for the focal elevation of synthesis of basic fibroblast growth factor and one of its high-affinity receptors (flg) following a localized cortical brain injury. The Journal of Neuroscience, *12*(10), 3828-3837.
- Lynch, G., Gall, C., & Cotman, C (1977). Temporal parameters of axon "sprouting" in the brain of the adult rat. Experimental Neurology, *54*, 179-183.

- Lynch, G., Rose, G., Gall, C., & Cotman, C. W. (1975). The response of the dentate gyrus to partial deafferentation. In M. Santini (Ed.), Golgi centennial symposium proceedings (pp. 505-517). New York: Raven Press.
- MacLusky, N. J., & Naftolin, F. (1981). Sexual differentiation of the nervous system. Science, 211, 1294-1303.
- Mandel, R. J., Gage, F. H., & Thal, L. J. (1989). Spatial learning in rats: Correlation with cortical choline acetyltransferase and improvement with NGF following NBM damage. Experimental Neurology, 104, 208-217.
- Mansour-Robaey, S., Clarke, D. B., Wang, Y. C., Bray, G. M., & Aguayo, A. J. (1994). Effects of ocular injury and administration of brain-derived neurotrophic factor on survival and regrowth of axotomized retinal ganglion cells. Proceedings of the National Academy of Sciences, USA, 91, 1632-1636.
- Matthews, D. A., Cotman, C. W., & Lynch, G. S. (1976a). An electron microscopic study of lesion-induced synaptogenesis in the dentate gyrus of the adult rat: I. Magnitude and time course of degeneration. Brain Research, 115, 1-21.
- Matthews, D. A., Cotman, C. W., & Lynch, G. S. (1976b). An electron microscopic study of lesion-induced synaptogenesis in the dentate gyrus of the adult rat: II. Reappearance of morphologically normal contacts. Brain Research, 115, 23-41.
- Mattson, M.P., Murrain, M., Guthrie, P. B., & Kater, S. B. (1989). Fibroblast growth factor and glutamate: Opposing roles in the generation and degeneration of hippocampal neuroarchitecture. The Journal of Neuroscience, 9, 3728-3732.
- McGlone, J. (1980). Sex differences in human brain asymmetry: A critical survey. Behavioral and Brain Sciences, 3, 215-263.
- Miller, L. C., & Cooper, R. M. (1974). Translucent occluders and the role of visual cortex in pattern vision. Brain Research, 79, 45-59.
- Miyamoto, O., Itano, T., Fujisawa, M., Tokuda, M., Matsui, H., Nagao, S., & Hatase, O. (1993). Exogenous basic fibroblast growth factor and nerve growth factor enhance sprouting of acetylcholinesterase positive fibers in denervated hippocampus. Acta Medica Okayama, 47(3), 139-144.

- Mohammed, A. K., Winblad, B., Ebendal, T., & Larkfors, L. (1990). Environmental influence on behaviour and nerve growth factor in the brain. Brain Research, 528, 62-72.
- Morris, R. G. M. (1981). Spatial localization does not require the presence of local cues. Learning and Motivation, 12, 239-260.
- Morrison, R. S., Sharma, A., DeVellis, J., & Bradshaw, R. A. (1986). Basic fibroblast growth factor supports the survival of cerebral cortical neurons in primary culture. Proceedings of the National Academy of Sciences, USA, 83, 7537-7541.
- Morse, J. K., Wiegand, S. J., Anderson, K., You, Y., Cai, N., Camahan, J., Miller, J., DiStefano, P. S., Altar, C. A., Lindsay, R. M., & Alderson, R. F. (1993). Brain-derived neurotrophic factor (BDNF) prevents the degeneration of medial septal cholinergic neurons following fimbria transection. The Journal of Neuroscience, 13, 4146-4156.
- Nadler, J. V., Cotman, C. W., & Lynch, G. S. (1977). Histochemical evidence of altered development of cholinergic fibers in the rat dentate gyrus following lesions: I. Time course after complete unilateral entorhinal lesion at various ages. The Journal of Comparative Neurology, 171, 561-588.
- Needels, D. L., & Cotman, C. W. (1988). Basic fibroblast growth factor increases the survival of rat dentate granule cells in culture. Society of Neurosciences Abstracts, 14, 363.
- Nieto-Sampedro, M., & Cotman, C. W. (1985). Growth factor induction and temporal order in central nervous system repair. In C. W. Cotman (Ed.), Synaptic plasticity (pp.407-456). New York: Guilford Press.
- Nieto-Sampedro, M., Lewis, E. R., Cotman, C. W., Manthorpe, M., Skaper, S. D., Barbin, G., Longo, F. M., & Varon, S. (1982). Brain injury causes a time-dependent increase in neurotrophic activity at the lesion site. Science, 221, 860-861.
- Nieto-Sampedro, M., Manthorpe, M., Barbin, G., Varon, S., & Cotman, C. W. (1983). Injury-induced neurotrophic activity in adult rat brain: Correlations with survival of delayed implants in the wound cavity. The Journal of Neuroscience, 3, 2219-2229.

- Nieto-Sampedro, M., Saneto, R. P., deVellis, J., & Cotman, C. W. (1985). The control of glial populations in brain: Changes in astrocytic mitogenic and morphogenic factors in response to injury. Brain Research, 343(2), 320-328.
- Nordeen, E. J., & Yahr, P. (1992). Hemispheric asymmetries in the behavioral and hormonal effects of sexually differentiating mammalian brain. Science, 218, 391-394.
- Otto, D., Frotscher, M., & Unsicker, K. (1989). Basic fibroblast growth factor and nerve growth factor administered in gel foam rescue medial septal neurons after fimbria-fornix transection. The Journal of Neuroscience, 22, 83-91.
- Otto, D., & Unsicker, K. (1990). Basic FGF reverses chemical and morphological deficits in the nigrostriatal system of MPTP-treated mice. The Journal of Neuroscience, 10, 1912-1921.
- Paxinos, G., & Watson, C. (1986). The rat brain in stereotaxic coordinates (2nd ed.). San Diego, CA: Academic Press, Inc.
- Peters, A., & Feldman, M. L. (1976). The projection of the lateral geniculate nucleus to area 17 of the rat cerebral cortex. I. General description. Journal of Neurocytology, 5, 63-84.
- Pruss, R. M., Bartlett, P. F., Gavrilovic, J., Lisak, R. P., & Rattray, S. (1982). Mitogens for glial cells: A comparison of the response of cultured astrocytes, oligodendrocytes, and Schwann cells. Developmental Brain Research, 2, 19-35.
- Riva, M. A., Gale, K., & Mocchetti, I. (1992). Basic fibroblast growth factor mRNA increases in specific brain regions following convulsive seizures. Molecular Brain Research, 15, 311-318.
- Rosenzweig, M. R., Bennett, E. L., & Diamond, M. C. (1972). Chemical and anatomical plasticity of brain: Replications and extensions, 1970. In J. Gaito (Ed.), Macromolecules and behavior (pp. 205-277). New York: Appelton.
- Schade, J. P., & Baxter, C. F. (1960). Changes during growth in the volume and surface area of cortical neurons in the rabbit. Experimental Neurology, 2, 158-178.

- Schallert, T. (1983). Sensorimotor impairment and recovery of function in brain-damaged rats: Reappearance of symptoms during old age. Behavioral Neuroscience, *97*, 159-164.
- Shirokawa, T., & Kasamatsu, T. (1986). Concentration-dependent suppression by B-adrenergic antagonists of the shift in ocular dominance following monocular deprivation in kitten visual cortex. Neuroscience, *18*, 1035-1046.
- Sievers, J., Hansmann, B., Unsicker, K., & Berry, M. (1987). Fibroblast growth factors promote the survival of adult rat retinal ganglion cells after transection of the optic nerve. Neuroscience Letters, *76*, 157-162.
- Singh, D. (1973). Effects of preoperative training on food-motivated behavior of hypothalamic hyperphagic rats. Journal of Comparative and Physiological Psychology, *84*, 47-52.
- Singh, D. (1974). Role of preoperative experience on reaction to quinine taste in hypothalamic hyperphagic rats. Journal of Comparative and Physiological Psychology, *86*, 674-678.
- Sirevaag, A. M., & Greenough, W. T. (1987). Differential rearing effects on rat visual cortex synapses. III. Neuronal and glial nuclei, boutons, dendrites, and capillaries. Brain Research, *424*, 320-332.
- Sohlberg, M. M., & Mateer, C. A. (1989). Introduction to cognitive rehabilitation. New York: Guilford Press.
- Stein, D. G., Finger, S., & Hart, T. (1983). Brain damage and recovery: Problems and perspectives. Behavioral and Neural Biology, *37*, 185-222.
- Stein, D. G., & Firl, A. C. (1976). Brain damage and reorganization of function in old age. Experimental Neurology, *52*, 157-167.
- Stewart, J., & Kolb, B. (1988). The effects of neonatal gonadectomy and prenatal stress on cortical thicknesses and asymmetry in rats. Behavioral and Neural Biology, *49*, 344-360.
- Sutherland, R. J., Kolb, B., Becker, J. B., & Wishaw, I. Q. (1982). Cortical noradrenaline depletion eliminates sparing of spatial learning after neonatal frontal cortex damage in the rat. Neuroscience Letters, *32*, 125-130.

- Sutherland, R. J., Wishaw, 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.
- Takami, K., Iwane, M., Kiyota, Y., Miyamoto, M., Tsukuda, R., & Shiosaka, S. (1992). Increase of basic fibroblast growth factor immunoreactivity and its mRNA level in rat brain following transient forebrain ischemia. Experimental Brain Research, 90(1), 1-10.
- Teuber, H.-L. (1975). Recovery of function after brain injury in man. In Outcome of severe damage to the nervous system. Ciba Foundation Symposium 34. Amsterdam: Elsevier/North Holland.
- Turner, A. M., & Greenough, W. T. (1985). Differential rearing effects on rat visual cortex synapses. I. synaptic and neuronal density and synapses per neuron. Brain Research, 329, 195-203.
- Vargha-Khadem, F., Watters, G., & O'Gorman, A. M. (1985). Development of speech and language following bilateral frontal lesions. Brain and Language, 37, 167-183.
- Vlodavsky, I., Bar Shavit, R., Ishai Michaeli, R., Bashkin, P., & Fuks, Z. (1991). Extracellular sequestration and release of fibroblast growth factor: A regulatory mechanism? Trends in Biochemistry, 16, 268-271.
- Walicke, P. A. (1988). Basic and acidic fibroblast growth factors have trophic effects on neurons from multiple CNS regions. The Journal of Neuroscience, 8, 2618-2627.
- Walicke, P., Cowan, W. M., Ueno, N., Baird, A., & Guillemin, R. (1986). Fibroblast growth factor promotes survival of dissociated hippocampal neurons and enhances neurite extension. Proceedings of the National Academy of Sciences, USA, 83, 3012-3016.
- Wanaka, A., Johnson, E. G., Jr., & Milbrandt, J. (1990). Localization of FGF receptor mRNA in the adult rat central nervous system by in situ hybridization. Neuron, 5, 267-281.
- West, R. W., & Greenough, W. T. (1972). Effect of environmental complexity on cortical synapses of rats: Preliminary results. Behavioral Biology, 7, 279-284.

- Whishaw, I. Q. (1990). The decorticate rat. In B. Kolb, & R. C. Tees (Eds.), The cerebral cortex of the rat (pp.239-267). Cambridge: MIT Press.
- Whishaw, I. Q., & Kolb, B. (1989). Tongue protrusion mediated by spared anterior ventrolateral neocortex in neonatally decorticate rats: Behavioral support for the neurogenetic hypothesis. Behavioral Brain Research, 32, 101-113.
- Whishaw, I. Q., O'Connor, R. B., & 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.
- Whishaw, I. Q., Pellis, S. M., & Gorny, B. P. (1992). Medial frontal cortex lesions impair the aiming component of rat reaching. Behavioral Brain Research, 50, 93-104.
- Whishaw, I.Q., Zaborowski, J. A., & Kolb, B. (1984). Post surgical enrichment aids adult hemidecorticated rats on a spatial navigation task. Behavioral and Neural Biology, 42, 183-190.
- Widmer, H. R., Knusel, B., & Hefti, F. (1993). BDNF protection of basal forebrain cholinergic neurons after axotomy: Complete protection of p75NGFR-positive cells. Neuroreport, 4, 363-366.
- Will, B. (1981). The influence of environment on recovery after brain damage in rodents. In M. W. van Hof, & G. Mohn (Eds.), Functional recovery from brain damage (pp. 167-188). Amsterdam: Elsevier/North Holland.
- Will, B. E., Kelche, C. R., & Deluzarche, F. (1981). Effects of postoperative environment on functional recovery after entorhinal cortex lesions in the rat. Behavioral and Neural Biology, 33, 303-316.
- Woods, B. T. (1980). The restricted effects of right hemisphere lesions after age one; Wechsler test data. Neuropsychologia, 18, 65-70.
- Wooley, C. S., Gould, E., Frankfurt, M., & McEwen, B. S. (1990). Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. The Journal of Neuroscience, 10, 4035-4039.
- Yoshida, K., & Gage, F. H. (1991). Fibroblast growth factors stimulate nerve growth factor synthesis and secretion by astrocytes. Brain Research, 538, 118-126.

Yoshida, K., & Gage, F. H. (1992). Cooperative regulation of nerve growth factor synthesis and secretion in fibroblasts and astrocytes by fibroblast growth factor and other cytokines. Brain Research, 569, 14-25.

Zilles, K. (1985). The cortex of the rat: A stereotaxic atlas. Berlin Heidelberg: Springer Verlag.

APPENDIX

Immunohistochemistry illustrating expression of bFGF in the injured rat brain.

