

**MECHANISMS UNDERLYING RECOVERY FROM
EARLY CORTICAL INJURY IN RATS**

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Abstract

Previous work has shown that removal of the midline frontal cortex at seven to ten days of age is followed by recovery of function correlated with apparent spontaneous generation of new tissue in the lesion cavity. The question asked in the present thesis was whether the removal of the regrown tissue in adulthood would block normal function. Rats that received P10 frontal lesions underwent second lesions at P160, and were compared to rats with only P10 or P160 lesions. Rats with P10 + P160 lesions were severely impaired on a spatial learning task, especially relative to the P10 lesion-only rats. In a second experiment, rats with P10 + P160 lesions were given intra-ventricular infusions of a cocktail of three growth factors. The animals with growth factors showed marked behavioral recovery, although there was no cell regeneration. The results of these experiments suggest that filled-in tissue in neonatally lesioned rats is functional.

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List of Abbreviations

Basic Fibroblast Growth Factor.....	bFGF
Central Nervous System.....	CNS
Epidermal Growth Factor.....	EGF
Neurotrophic Factor.....	NTF
Nerve Growth Factor.....	NGF
Peripheral Nervous System.....	PNS
Subventricular Zone.....	SVZ

Chapter 1. General Introduction

As the “Decade of the Brain” (1990-2000) draws to a close, it seems appropriate to review some of the advances in the area of brain recovery from injury. In humans, brain dysfunction as a result of trauma or degeneration is manifested behaviorally in many forms including motor, speech, or perceptual difficulties, cognitive impairment, and personality changes. Statistics from the NeuroScience Network indicate that 120,000 Canadians will suffer a stroke or closed head injury this year, and in fact, brain injury is a leading cause of death among Canadians. Aside from traumatic brain injury such as accidents, brain injury also encompasses organic trauma such as aneurism, and degenerative disorders such as Alzheimer’s disease and Parkinson’s disease. The effects of brain damage costs Canadians millions of dollars in lost wages, the health care system millions in treatment, and most importantly, may cost the patient the quality of life to which he or she was accustomed.

Advances in medical technology have extended the average Canadian’s life expectancy, and as such, we see that the natural aging process is accompanied by brain degeneration or dysfunction. It is imperative that the research on recovery from brain injury be a forefront of science, not only for the patients that suffer, but for their families as well.

Recovery following insult to the brain has long been regarded as hopeless, but in fact, the brain is capable of remarkable recovery that, until assisted by recent advances in

technology, was not detected. It is the intention of the following series of experiments to bring the possibilities of recovery to light by examining both the ability of the brain to replace lost tissue, and the reparative effect of directing endogenous chemicals to injured tissue.

Background

Until less than 200 years ago the structure of the nervous system remained elusive, due in part to the lack of optical instruments to examine it. The light microscope of the 1820's launched the cellular examination of the nervous system. Unfortunately, nervous tissue is difficult to visualize in the microscope, and rapidly deteriorates when unfixed. In the early part of the 1800's, a young doctor named Camillo Golgi made a significant contribution to the study of the nervous system with the development of his staining method, today known as the Golgi stain. Although it has been refined repeatedly since the advent of the stain, Golgi spent years developing a method for hardening and coloring the tissue so that it was visible in the microscope. The stain consisted of impregnating the nervous tissue with silver nitrate, of which the silver remained on the cell's surface and revealed the finest complexity of the cell's exterior structures.

In the 1880's, an anatomist named Santiago Ramon y Cajal had the opportunity of observing the tissue resulting from Golgi's stain, and immediately set about with a series of experiments that revolutionized the study of the nervous system (Oliverio & Shepherd, 1991). Cajal postulated that changes occur amongst neurons when learning takes place

(Ramon y Cajal, 1933). In the 1940's, a Polish scientist by the name of Jerzy Konorski suggested two types of changes that impact neurons and their connections, the synapses. The first is a transitory change, that is, one that is a temporary and unstable change in the excitement of a neuron. The second is an enduring, or permanent change, which may represent a morphological or functional change, of which learning a new language is a prime example.

To answer the question: "which element of the cell actually changes?", D.O. Hebb, a Canadian psychologist, suggested looking to the activity of the synapse. Hebb argued that synaptic change is an ongoing event occurring during development, learning, and aging (Hebb, 1949). Ramon y Cajal's proposal in the late 1920's led the scientific community to believe that plasticity took place at the level of the synapse, and Hebb's posited mechanism in the 1940's supported the claim.

Today we believe there are two possible mechanisms for change in the brain: the reorganization of old circuits in the brain, or the creation of novel circuits. New circuits may be composed of old cells or new cells, but in both cases it is synaptic organization that is the key to behavioral change. In the latter case, it may also involve the generation of new neurons.

Brain Plasticity and Recovery From Brain Damage

In mammalian species, it appears that the brain is most plastic during development, both with respect to cell generation and synaptic change, but elements of

plasticity do continue in adulthood. For example, learning a new language in adulthood is considered more difficult than in childhood (Werker & Tees, 1999). However, when an adult does acquire the new language skills, the process by which he learned the language presumably causes permanent changes in the structure and function of cortical areas, just as does original language learning. Neurogenesis, the formation of new neurons, is not common in adult mammals. For years, the scientific community has firmly held the belief that connections in the brain are permanent, and mammalian neurogenesis is limited to development. This longstanding doctrine was supported by Rakic's work with rhesus macaque monkeys, in which, using a tritiated thymidine labelling method, he found no postnatal neurogenesis (Rakic, 1985). These data were generalized to humans and as a result, most researchers abandoned the possibility of recovery of neurons following CNS damage. Nonetheless, there are several lines of evidence suggesting that neurogenesis is possible in the post-mitotic brain ¹.

Research in the 1970's revealed the possibility of cortical neurogenesis in adult birds, something previously unheard of. In fact, until that time, it was believed that only cold-blooded animals and invertebrates displayed cortical neurogenesis in adulthood. Neurogenesis is known to persist in the central nervous systems of fish and reptiles, in

¹ In Shankle et al (1998), the authors argue against the validity of Rakic's conclusions; and refer to a series of experiments by Conel, which they suggest offers an accurate estimate of the changes in numbers of cortical neurons in humans (Conel, 1939; Conel, 1941; Conel, 1947; Conel, 1951; Conel, 1955; Conel, 1959; Conel, 1963; Conel, 1967).

which both the brains and bodies continue to grow following sexual maturity (Birse, Leonard, & Coggeshall, 1980; Garcia-Verdugo et al., 1989; Lopez-Garcia, 1993). Evidence of abundant cortical neurogenesis in the song control nucleus of the adult avian brain first appeared in 1985 (Goldman & Nottebohm, 1985). Since that time, other reports of neurogenesis in adults of many bird species have followed, including zebra finches, parakeets, doves, quails, and chickens (Nottebohm, 1985). It appears that neurogenesis in avian species follows a pattern similar to that of development: the neurons migrate from the sub-ventricular zone of the brain until they assume their final position (Nottebohm & Alvarez-Buylla, 1993).

The subventricular zone (SVZ) of the brain is an area known to be rich in stem cells. Stem cells are defined as cells that have the ability to proliferate; exhibit self-maintenance or renewal, generate many progeny, and produce new cells in response to injury (Weiss et al., 1996). Stem cells are not exclusive to the brain, they are also found in bone marrow, skin cells, and intestinal epithelium, among others. Weiss et al (1996) describe the proliferation of brain stem cells, both *in vivo* in mice, and *in vitro*, and suggest the findings to be evidence for neurogenesis in adult mammals.

The notion of cortical neurogenesis in adult mammals dates back as far as a 1963 publication in which Altman reports neurogenesis in some areas of adult rat and cat brains (Altman, 1963). Further credibility of adult neurogenesis is provided by Altman's 1970 publication, in which he presents data of neurogenesis in the dentate gyrus and in the olfactory bulb. As a result of these data, the possibility of neurogenesis in adult and

juvenile rats is widely accepted (Bayer, 1983; Bayer, 1985; Bayer, Yackel, & Puri, 1982; Gould, Cameron, Daniels, Wooley, & McEwan, 1992; Gould, Reeves, & Gross, 1999; Kaplan & Bell, 1984; Trice & Stanfield, 1986). Recently, Gould (1999) reported the addition of neurons in adulthood in primate neocortex. In her study, newly formed cells were detected in the subventricular zone of adult animals, and these cells were seen to migrate through existing white matter and then differentiate in neocortical regions (Gould et al., 1999).

Factors affecting recovery following brain damage

There are four factors inhibiting the regeneration of CNS neurons: lack of endoneurial sheaths, lack of growth stimuli, production of growth inhibitors, and formation of scar tissue (Brown, Hopkins, & Keynes, 1991). Endoneurial sheaths are fibres that serve to guide regenerating axons in the PNS, and appear to exist only in the peripheral nervous system (PNS). Regeneration in the PNS is enabled by Schwann cells surrounding neurons, which allow macrophages to penetrate neurons, to provide factors that aid in regeneration. Conversely, the CNS does not contain Schwann cells but oligodendrocytes that produce substances that actually retard the regeneration of axons (Bach-y-Rita & Bach-y-Rita, 1990; Brown et al., 1991).

Research in the area of recovery from brain damage has uncovered some factors that can be manipulated to optimize the brain's reaction to injury, and possibly promote the functional recovery from the injury. Some of the factors affecting the behavioral

outcome following brain damage include the age at which the lesion occurred, the location of the damage, the environment the animal is exposed to, and finally, endogenous chemicals known as neurotrophins. When any or all of these factors are manipulated, the outcome for the animal is improved over control animals. Therefore, the possibility remains that treatment following brain damage has the potential to improve the behavioral outcome of the animal.

Age effects

One pioneer of brain research, Margaret Kennard, is known for her conclusions that brain damage has a much more favorable outcome in youth than in old age (Kennard, 1942). Kennard worked extensively with primates, and observed that younger animals were more likely to recover from cortical damage than older individuals with similar damage. For instance, in her 1936 publication, Kennard notes that those adult monkeys that underwent removal of the entire motor area were slower to recover than very young or adolescent animals (Kennard, 1936). The idea that young animals are more apt to recover than older animals has been termed the "Kennard Effect", after Kennard's observations ².

Evidence of detrimental effects of very young lesions is presented by Kolb et al (1987). The author reports that rats lesioned at one to five days of age show a poor

²Finger (1988), disputes the term "Kennard Effect", and argues that the idea of young animals recovering more rapidly than older animals is not a concept attributable to Kennard, but rather to her predecessors, namely Vulpian, Soltmann, and Weed.

behavioral outcome, and a brain smaller than control littermates. However, in contrast to the very young animals, rats lesioned at seven to ten days of age show remarkable recovery and considerable sparing of function when tested in adulthood (Kolb & Whishaw, In press). In fact, it appears as though animals lesioned at seven to ten days of age show nearly complete functional recovery in terms of compensation for the injury. This research serves to illustrate the importance of critical periods, sensitive times of development where the environment plays a great role. If animals are lesioned during the critical period of brain development, from postnatal day one to five, the animal suffers behavioral deficits, and anatomical effects such as a smaller and lighter brain (Kolb & Whishaw, 1981; Stewart & Kolb, 1988). It has been shown that animals lesioned during the seven to twelve day period demonstrate functional recovery, which can be attributed to the brain undergoing a period of intense synaptic formation, dendritic growth, and possibly neurogenesis (Kolb, Gibb, & van der Kooy, 1994; Kolb, Ladowski, Gibb, & Gorny, 1996b).

Although Kennard's principle suggests that the younger the animal, the better the outcome, such is not the case based on research by Kolb et al (1996c). The authors report that rats lesioned at one to five days of age show a poor behavioral outcome, and a brain smaller than control littermates. However, in contrast to the very young animals, rats lesioned at seven to ten days of age show remarkable recovery and considerable sparing of function when tested in adulthood. In fact, it appears as though animals lesioned at seven to ten days of age show nearly complete recovery in terms of

compensation for the injury. In human age, a rat at the age of postnatal day seven is approximately equivalent to a human at one month.

Lesion site

Aside from the age at which the lesion occurred, other factors in recovery include the size and site of the insult. A large lesion is less likely to support recovery, relative to a smaller lesion (Kennard, 1936). Laterality is another factor affecting behavioral outcome. A lesion on one side of the brain, called a unilateral lesion, generally has a better functional outcome than a bilateral lesion. Thus, the restriction of damage to one hemisphere is considered to be an influential element to support recovery. For example, young children with complete hemispherectomies may show complete recovery, and in adulthood their cognitive abilities may even exceed those of their intact-brain peers (Smith & Sugar, 1975). In adulthood, however, unilateral lesions may have serious consequences because the mature human brain is organized with specialized centres that are found only on one side of the brain. This cerebral asymmetry may pose serious problems for functional recovery, for instance, damage to the brain in the left posterior temporal region may result in difficulty producing or understanding language. Cerebral asymmetry and lesion size are not mutually exclusive, however. A small bilateral lesion has more negative behavioral effects than a larger unilateral lesion (Kolb, Zaborowski, & Whishaw, 1989).

Environmental effects

The experiences of animals has a great impact on the recovery from brain damage. In humans, for example, it is common practice to tactilely stroke premature babies, resulting in earlier hospital discharge (Field et al., 1986; Schanberg & Field, 1987; Solkoff & Matuszak, 1975). It is presumed that the tactile stimulation results in enhanced brain development. To test this hypothesis in rats, Kolb et al (unpublished observations) stroked rat pups with a small paintbrush, and found an increase in cortical thickness and dendritic arborization. In separate experiments, rats were encouraged to interact with a complex environment. Rats are normally housed in small cages, but were group housed in an "enriched environment" which consisted of group housing, and exposure to a rotating series of toys and playthings. The rats were not fed through the traditional hopper, but rather were exposed to food in novel ways, such as scattered or hanging, for which they may have had to climb or problem solve. One of the most impressive findings of this line of research is the discovery that rats placed in the enriched environment after brain damage show more complete functional recovery, thicker cortices, and greater dendritic arborization than cage-housed littermates (unpublished observations). Even in senescence, animals are capable of plastic changes to the brain, and these changes are revealed in increased dendrite length and branching, or more spines, putatively implicating an increase in synapses. It appears, then, that the brain is able to change structurally even in old age. It follows that one would question the ability of the brain to repair itself in adulthood. Is it the case that new connections

are made to replace lost ones, or is the brain actually able to regrow lost tissue?

Neurotrophins and Recovery of Function

Recent studies have shown that it may indeed be possible to stimulate the brain to generate new neurons. Kolb et al have shown that the midline frontal cortex and olfactory bulb can spontaneously regenerate if they are selectively removed around postnatal day 10 (e.g. Kolb, Gibb, Gorny, & Whishaw, 1998b; Kolb et al., 1996c; Kolb & Whishaw, In press). In contrast, this spontaneous regrowth is not normally seen in adult rats. Nonetheless, through an exhaustive series of experiments utilizing various endogenous chemicals (neurotrophic factors), Kolb and colleagues have been able to stimulate the filling-in of tissue in adult rats with cortical removals (Kolb, Cote, Ribeiro-da-Silva, & Cuello, 1997a; Kolb, Cote, Ribeiro-da-Silva, & Cuello, 1997b; Kolb, Gorny, S, Ribeiro-Da-Silva, & Cuello, 1997c). Neurotrophic factors (NTF's) are chemicals that promote the growth and survival of neurons and are defined as polypeptides that are necessary for the continued survival of specific populations of neurons in both development and adulthood (Henderson, 1996). Although NTF's can be added to the injured brain experimentally to promote recovery, (e.g.: Barde, 1989; Cuello, Maysinger, & L.Garofalo, 1992; Kolb et al., 1997a), they appear to be present in a normal brain as well. Endogenous NTF's appear to regulate the survival and differentiation of target cells during embryonic life, and also maintain their specific function in adulthood (Gomez-Pinilla & Cotman, 1992). Aside from their role in development, NTF's likely play a role

in plastic changes throughout life; those involved in modifying neural circuits, and maintaining others (Kolb et al., 1997c). The limited amount of growth factors found in adult animals is one of the putative reasons that animals do not recover as well in adulthood as in youth. Recent studies suggest that the administration of some NTF's may stimulate recovery from brain damage.

Nerve growth factor (NGF) has been found to reverse anatomical and behavioral effects of lesions in cortical pyramidal cells (Kolb et al., 1997a; Kolb et al., 1997b; Kolb et al., 1997c), as well as stimulating growth in a number of cells including noradrenergic neurons (Bjorklund & Stenevi, 1972), cholinergic neurons (Hefti, Dravid, & Hartikka, 1984; Kromer, 1987), and Purkinje cells of the cerebellum (Cohen-Corey, Dreyfus, & Black, 1991). The mechanism supporting functional recovery after treatment with NGF is possibly the stimulation of remaining cortical neurons not destroyed or damaged, though some findings suggest that exogenous administration of NGF can induce axonal sprouting (Chen, Masliah, Mallory, Terry, & Gage, 1995; Gage, Tuszynski, Yoshida, & Higgins, 1991; Garofalo & Cuello, 1992) and synaptogenesis (Chen et al., 1995; Garofalo & Cuello, 1992).

Basic fibroblast growth factor (bFGF), another neurotrophic factor, belongs to a class of factors that have mitogenic effects on fibroblasts, endothelial cells, and other cells of mesenchymal origin (Folkman & Klagsbrun, 1987; Gospodarowicz, Neufeld, & Schwiegerer, 1986; Thomas, 1987). In the normal brain, bFGF is found in both neurons and glia, as well as the meninges and ependymal cells of the ventricular system (Logan &

Berry, 1993). Basic fibroblast growth factor appears to play an important role in the trophic response to brain injury, for example it encourages the production of other proteins, one of which is NGF. An increased amount of bFGF found in the brain is thought to be correlated with recovery following cortical injury, based on evidence of the factor having a potent effect on the survival and growth of CNS neurons (Eccleston & Silberberg, 1985; Gospodarowicz et al., 1986; Morrison, Sharma, DeVellis, & Bradshaw, 1986). There is also evidence to suggest that injury-induced bFGF promotes recovery that is observed in increase dendritic branching and spine density, similar to what recovery is seen in intact brains (Rowntree, 1995).

Taken together, the evidence suggests that NGF and bFGF may play an important role in stimulating recovery after cortical injury, largely by stimulating the reorganization of existing connections.

Evidence that synaptic change does occur after lesion

Following a lesion of the cortex, it is not unusual to see an adjacent area of the brain take over the function of the lesioned area (Kolb, Cioe, & Muirhead, 1998a; Kolb, Stewart, & Sutherland, 1997d). One way to quantify the change is to measure the number of synapses on a neuron. Although it is nearly impossible to count every single synapse, it is possible to estimate the number of synapses with a good degree of accuracy. Synapse number can increase either by the formation of more spines along a constant length of dendrite, or can increase by maintaining a constant number of spines

along an increased length of dendrite. Either way, there are more synapses, and thus, more contact sites for neighbouring cells, as the following model indicates.



One legitimate place to look for change in the remaining cortex is in tissue proximate to the lesion (Kolb et al., 1998a; Kolb et al., 1997d). That is, a lesion of motor cortex will likely not lead to changes in visual cortex. Change in adjacent cortex is not limited to a response to a lesion, however, and increases in spine density and dendrite length in parietal area 1, layer III pyramidal cells have also been observed following exposure to enriched environments and to certain drugs (Kolb, 1999; Kolb et al., 1997d; Kolb & Whishaw, 1998; Kolb & Whishaw, In press; Robinson & Kolb, 1999).

The use of NTF's in the injured brain, particularly NGF and bFGF, has shown changes in the adjacent areas of the brain, including increased spine density or dendritic length (Kolb et al., 1997a; Kolb et al., 1997c; Rowntree & Kolb, 1997). These changes are associated with functional recovery of animals with cortical removals in adulthood, thus providing evidence for the role of NTF's in recovery following brain injury.

Does neurogenesis occur in adult mammals?

As previously mentioned, Gould and colleagues appear to have identified neurogenesis in primate neocortex (Gould et al., 1999). Rat models have also suggested neurogenesis in the injured adult brain, particularly when treated with epidermal growth factor (EGF). EGF, another neurotrophin, is usually found in the brain during development. When EGF is infused into the lateral ventricles of the brain, however, it appears to promote the migration of stem cells through the rostral stream, and they appear to differentiate into neurons in the olfactory bulb (Goldman & Nottebohm, 1985).

Why do experiments involving the brain often utilize rats?

Rodent models are the most common models of brain damage and are useful in pinpointing the types of deficits encountered following brain damage for three main reasons: (1) rat behavior is consistent and readily observable; (2) the animals can learn tasks quickly; and (3) a rat's lifespan is short enough to observe the stages of development through aging. Experiments using rats appear in scientific literature for a number of different types of studies, but in particular I will discuss animals used for the study of recovery following cortical lesions. Whishaw et al (1983) have suggested using empirical data, that is, relying on experience and observations to establish meaningful generalizations about rat behavior, and have recommended a battery of tests for rats. The tasks of the neuropsychological battery suggested are too numerous to review here, but some of the observations include: appearance of the animals, sensory and sensorimotor behavior, posture and immobility, and movement. The results of this type of objective

testing provides investigators with a series of behavioral observations that are useful in assessing the results of brain lesions or treatments. For instance, Kolb and colleagues have shown that rats with frontal lesions have deficits in performance of tests requiring spatial navigation, such as the Morris water task (Kolb et al., 1997a; Kolb et al., 1996a; Kolb et al., 1997c; Kolb et al., 1996c), and have been able to manipulate factors affecting the recovery of the rats. Similarly, Whishaw and colleagues have seen deficits in animals tested on skilled motor reaching tasks, such as the Whishaw reaching task (Whishaw, Dringenberg, & Pellis, 1992a; Whishaw et al., 1993; Whishaw, Pellis, & Gorny, 1992b) following certain lesions. These data can be valuable when applied to humans suffering from movement disorders, and the development of a treatment for such disorders as a result of research with rats is possible. In sum, valuable information can be gained from research with rats, and this knowledge may serve to explain the mechanisms behind recovery from brain damage.

Chapter 2. Rationale for Experiments

Although Kolb et al (e.g.: Kolb et al., 1998b; Kolb et al., 1996c) propose that neonatal rats are capable of spontaneous cortical regeneration following lesions, there are alternate explanations for the "regrowth" seen in seven to ten day old animals. Such suggestions include: 1) A collapse of nearby tissue, thereby filling in the cavity; 2) Scar tissue formed to fill in the cavity, or 3) a migration of cells that are not relevant to the expected function of the tissue. The current studies were designed to address these alternate explanations. In this series of experiments I chose: 1) to remove the regrown tissue in adult rats with medial frontal lesions on P10; and then, 2) to try to stimulate regeneration of brain tissue a second time by infusing NTF's.

In order to evaluate the function of the regrown tissue, both behavioral and anatomical analyses were conducted. The experiments focussed on functional recovery of performance on a behavioral task that is sensitive to lesions in medial frontal cortex, a spatial navigation task called the Morris water task.

Anatomical analyses consisted of various analyses including visual inspection of the lesion cavity, brain weight measures, and following Golgi-Cox processing, cortical thickness and dendritic analysis of nearby parietal area 1, layer III cells.

In sum, it is the intention of this series of experiments to show the relationship between filled-in tissue in the lesion cavity and functional recovery of the animal.

Chapter 3. Experiment One

Materials and Methods:

Subjects:

The study was done with 31 Long-Evans rats, derived from the Charles River Long-Evans strain. The distribution of animals per group was: control rats n=12, (6m, 6f), rats with postnatal day 10 lesions (P10) n=6, (3m, 3f), postnatal day 160 lesions (P160) n=5, (2m, 3f), and rats with combined postnatal day 10 and postnatal day 160 lesions (P10 + P160) n=6, (3m, 3f). See Figure 1 for the experimental design.

Surgical Procedures:

Day 10. All animals were operated on postnatal day 10 (P10). Ten animals received bilateral medial frontal lesions, and the remaining eighteen received sham lesions only, where the skin was excised and sutured. All animals were anaesthetized by cooling in a ThermoTron cooling chamber until their rectal temperatures were in the range of 18-20°C. The frontal bone was removed by cutting it with iris scissors and the cortical tissue was aspirated using a glass pipette. Once the operation was complete, animals were sutured with silk thread and slowly warmed under heat lamps to their normal body temperatures.

Day 160. Fifteen animals had medial frontal cortex aspiration lesions 150 days following the first surgery. Of these, 11 rats had P10 lesions, and five had sham lesions on P10. The animals were anaesthetized with sodium pentobarbital (females, 45mg/kg; males, 60

Experimental Design

Control



P10



P160



P10+P160



mg/kg), and placed in a stereotaxic instrument. The skull was drilled with a .5mm bit so as not to compromise the dura but to allow the entry of a rongeur tip. The frontal neocortex was exposed by removing the skull with the rongeurs from the bregmoidal junction anteriorly to the frontal bone suture, and laterally about 2mm from the midline on each side. After retraction of the dura, the medial frontal cortex was removed by glass pipette aspiration with the aid of a surgical microscope. The scalp was closed with stainless steel wound clips.

Behavioral Testing

Animals were tested on the Morris water task at approximately 200 days of age. Each of the animals was given eight trials per day, for five consecutive days. The tester was unaware of the grouping of any of the animals. The task consisted of a circular pool 180 cm in diameter, and contained 25 cm of water. The pool was painted white inside, and was filled with skim milk powder mixed with 20°C water. The milk aided the obstruction of the plexiglass platform located inside the SW quadrant of the pool. The platform's position remained the same throughout all the trials, except the final trial when the platform was removed in order to determine the swim area of the animals. The animals that were placed into the water hind feet first facing the side of the pool, at random designations of north, east, south, or west. The animals were permitted to swim for a maximum time of 60 seconds per trial. If a rat had not located the platform in that time, it was removed, and later subjected to another trial. If the platform was located, the

animals was allowed to remain on the dry platform for 10 seconds, then removed back to the transporting cage.

Swim latency was measured with the aid of a computer tracking system that followed the rats' black head in the white water. The program recorded swim latencies and swim distance for each animal on each of the 40 trials.

Quadrant Measure

On the 40th trial, the time allotted for finding the platform was reduced to 30 seconds, and the platform was removed from the pool. The animals were tracked by the computer, and the results of the amount of time spent in each quadrant was analyzed.

Anatomical Procedures

Following the completion of the behavioral testing, all animals were given an overdose of sodium pentobarbital, weighed, and intracardially perfused with 0.9% saline. In 30 of the animals, brains were removed, weighed, and placed in Golgi-Cox solution for 14 days. The brains were then transferred to 30% sucrose solution for a minimum of five days. Brains were sectioned at 200 μm on a vibratome, and mounted on 2% gelatin slides. The slides were stored in a humidity chamber until staining (following the procedure of Gibb & Kolb, 1998), then immediately coverslipped using Permount.

The remaining one animal that was not prepared for Golgi-Cox staining was perfused with 0.9% saline, followed by Lana's fix. This brain was stored in Lana's fix until it was cut to 50 μm sections, and some sections were then mounted on chromalum

slides for cresyl violet staining. The remaining free sections were stained using immunohistochemistry which consisted of GFAP, bFGF, and Nestin. Once stained, these sections were also mounted onto chromalum slides and coverslipped with Permount. The immunohistochemistry processes were intended to detect new astrocytes and new glia, which are not discernible using the Golgi-Cox method.

Brain Weight

In order to estimate the loss of brain tissue and possible new generation of brain tissue, the brains were weighed immediately following the removal from the skull. Before weighing, the spinal cord was cut even with the caudal edge of the cerebellum, the cerebellar paraflocculi were removed and the optic nerves were severed 1-2 mm posterior to the optic chiasm. In the analyses, brain weights must be considered from three aspects; the sex of the animals, type of lesion, and the age at which the lesion occurred.

Cortical Thickness

To measure cortical thickness, the Golgi-stained brain sections were projected on a Zeiss projector, set at a magnification of 13X. Measurements of cortical thickness were taken at a plane corresponding to 1 mm anterior to bregma, 2 mm posterior to bregma, and 6 mm posterior to bregma. Measurements were made at three different points in each of the planes of each hemisphere (as demonstrated in Stewart & Kolb, 1988).

Cell morphology was determined by drawing representative cells using the camera lucida procedure. Selected cells were located in area Par 1, layer III, and selected randomly. The cell must not have been obscured by blood vessels or other artifacts. The cell was magnified at 200X on a Zeiss Microscope, and then the image was traced onto paper by hand with the help of a drawing tube, which allows the drawer to visualize the cell and trace the cell simultaneously. Figure 1.1 is a representation of the apparatus used to draw cells. Five cells were drawn from each hemisphere of each animal, and these data were then analyzed for dendrite length using the method of Sholl (1956). This is a process in which concentric rings are overlaid on each cell tracing, and the apical dendrites and basilar dendrites are counted separately, recording the number of crossings of the dendrites per ring. Figure 1.2 illustrates the Sholl analysis rings.

Results

Behavioral Results

Latencies

Figures 2 and 3 clearly show that rats with medial frontal removal on P160, including both the P10+P160 and P160-only groups, were impaired relative to controls on the Morris water task (see Figures 2 and 3). The rats with P10 frontal lesions performed as well as controls.

A repeated-measures ANOVA (Group x Trial Block) indicated a significant effect of group ($F(3,27)=6.673$, $p<0.0018$), a significant effect of trial blocks ($F(9,27)=3.90$, $p<0.0001$), and a significant interaction between the two ($F(3,27)=1.645$,



Figure 1.1 **Representation of camera lucida
technique used to draw cells**

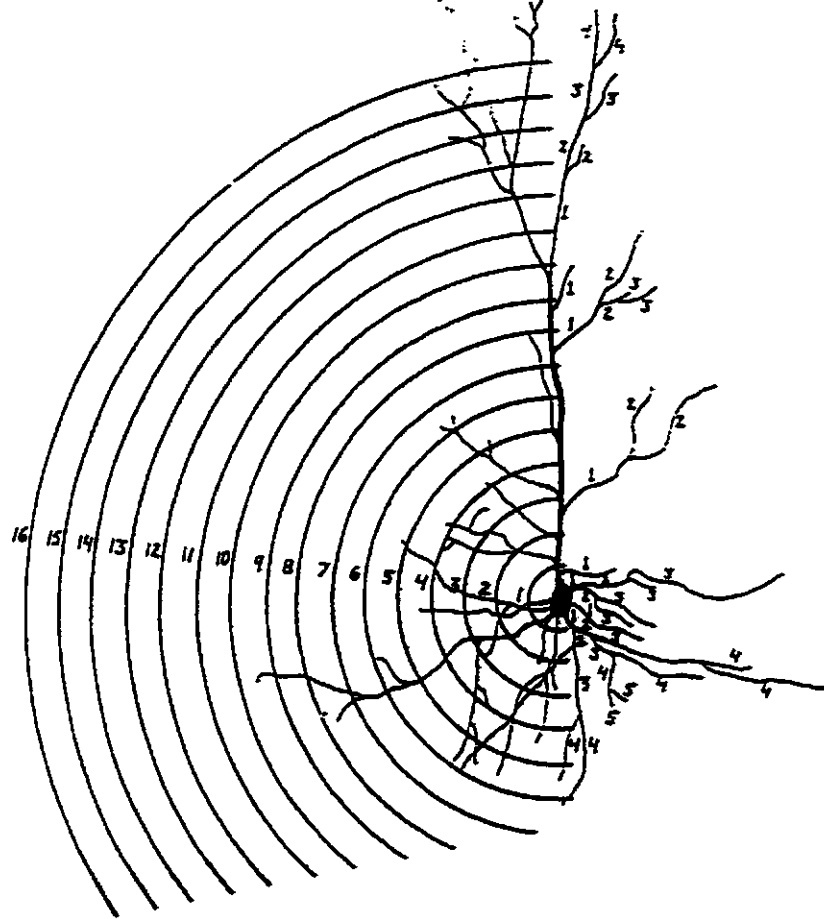


Figure 1.2 Example of Sholl ring overlaid atop a Par1, layer III cell

Place Task Performance

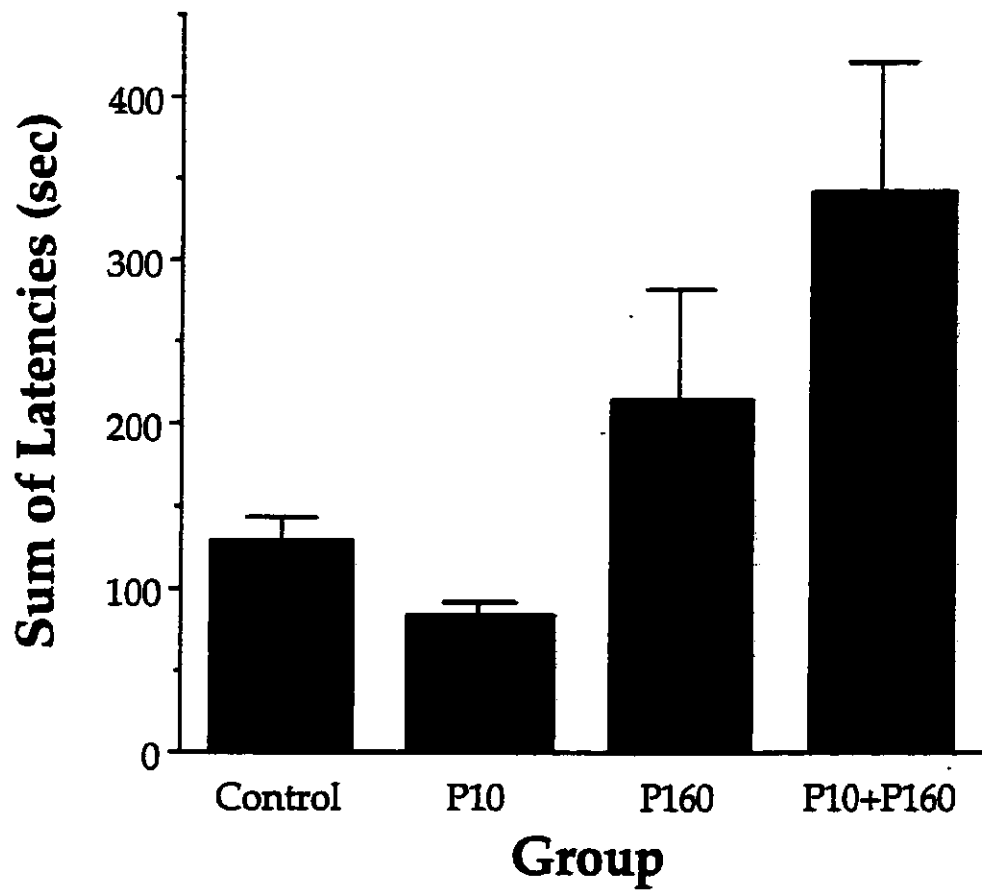


Figure 2. Sum of latencies of 10 trial blocks on Morris water task

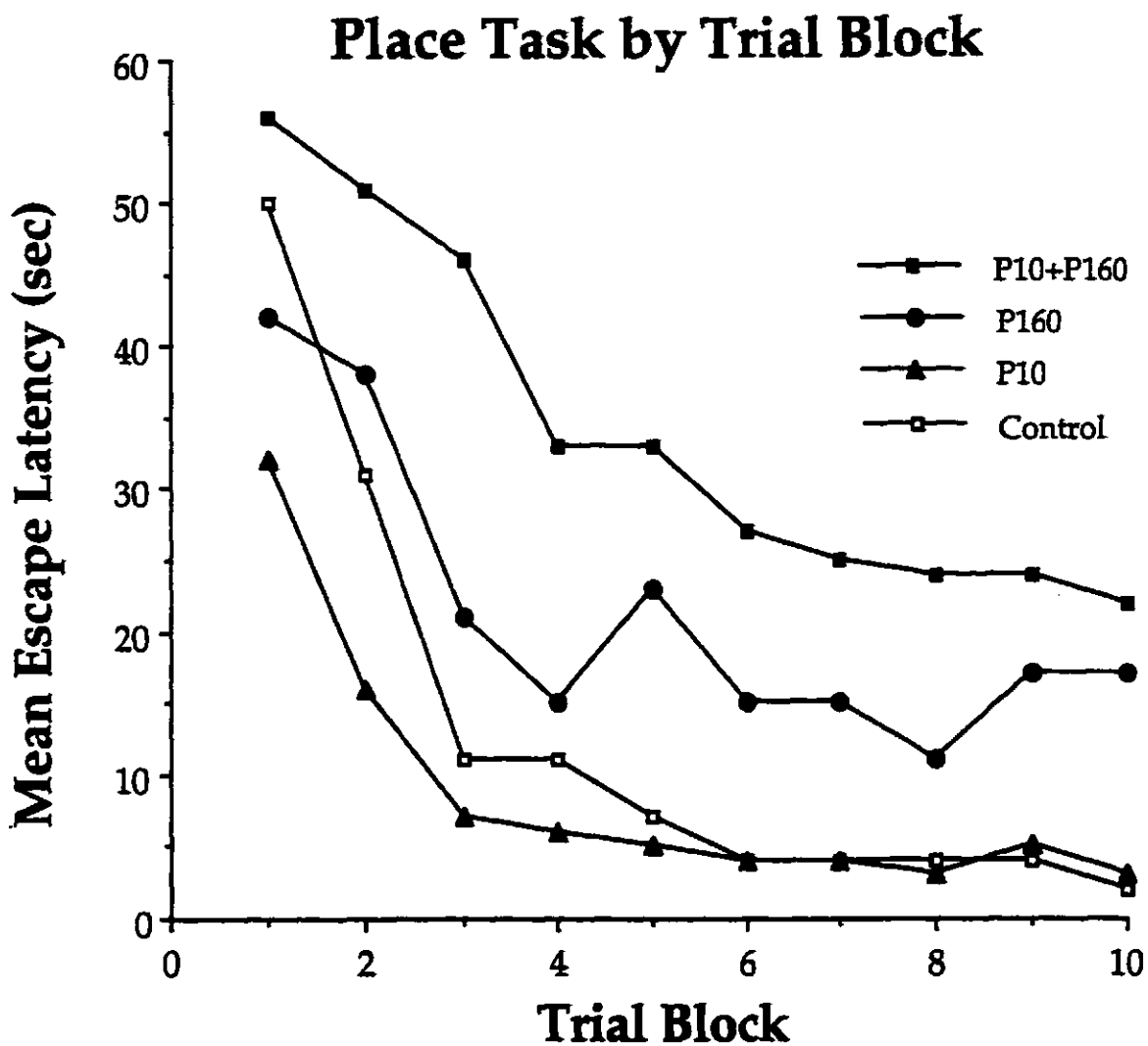


Figure 3. Latencies per trial block (4 trials/block) on Morris water task

$p < 0.0279$). These findings prompted an analysis of individual trial blocks, which showed that P10 and control groups are virtually indistinguishable by the 5th trial block (i.e., the 20th trial). In contrast, both the P160 and P10+P160 groups had significantly higher latencies on the task, and the P10 + P160 group were grossly impaired on the task (Figure 3). Post-hoc tests (Fisher's LSD) showed that the two P160 groups differed from the control group and P10 groups but not with one another. One-way ANOVA's on escape latency by trial block (TB) revealed significant between-group differences for trial block 1, ($F(3,25)=7.27, p < 0.0011$); TB 2, ($F(3,25)=4.69, p < 0.0099$); TB 3, ($F(3,25)=9.2, p < 0.0003$); TB 4, ($F(3,25)=3.55, p < 0.0288$); TB 5, ($F(3,25)=8.22, p < 0.0006$); TB 6, ($F(3,25)=4.18, p < 0.0157$); TB 7, ($F(3,25)=3.56, p < 0.0286$); TB 8, ($F(3,25)=3.6, p < 0.0274$); and TB 10, ($F(3,25)=3.01, p < 0.0492$)

Swim Distance

Figure 4 compares the overall swim distances of groups of subjects (collapsed across trial blocks). Overall, swim distances mirrored the group differences found in swim latencies. As before, the P10 group performed best on the task, and the adult lesion groups (P160 and P10+P160) were both impaired, relative to controls and to P10 animals. A one-way ANOVA indicated a main effect of group, ($F(3,24)=2.973, p=0.05$), and post-hoc tests (Fisher's LSD) revealed that the P160 and combined lesion groups were each different from the control group ($p < 0.05$), but were not different from each other.

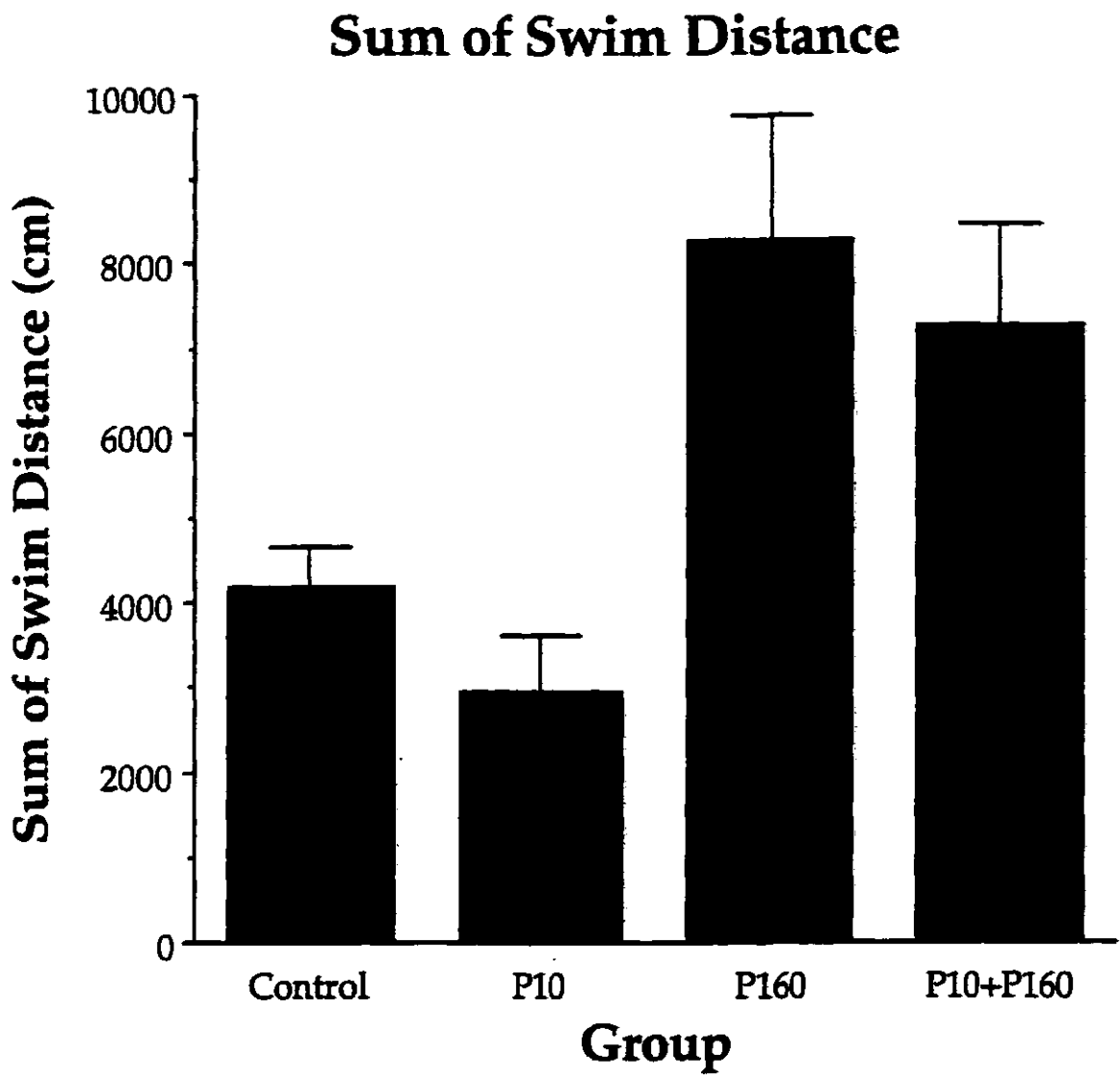


Figure 4. Sum of total swim distance for all trials of Morris water maze

Figure 5 illustrates group performance by trial block. A repeated-measures ANOVA found no significant group by swim distance interaction ($F(3,27)=0.673$, $p<.8891$), indicating that all groups' performance improved over time blocks. Significant differences were indicated between groups ($F(3,27)=2.923$, $p=0.05$) and among swim distances per trial block ($F(3,27)=46.406$, $p<0.0001$). These differences verify that swim distances were shorter for control and P10 groups relative to the groups that received lesions in adulthood.

Quadrant Measure

The quadrant measure indicates whether or not the animals have learned the location of the platform by the probe trial. An animal that has learned the task will spend the majority of the trial searching for the platform in the target quadrant. The overall results of dwell time per quadrant indicate that all of the groups had learned the task by the 40th trial. A repeated-measures ANOVA (Group x Quadrant) showed that all groups swam longer in the target quadrant than in the other quadrants ($F(3,72)=75.042$, $p<0.0001$). Figure 6 clearly illustrates this finding. No significant differences among groups was indicated ($F(3,24)=0.052$, $p=0.09$) nor was there a significant interaction of group by quadrant ($F(3,24)=1.712$, $p=.10$).

Place Task Performance

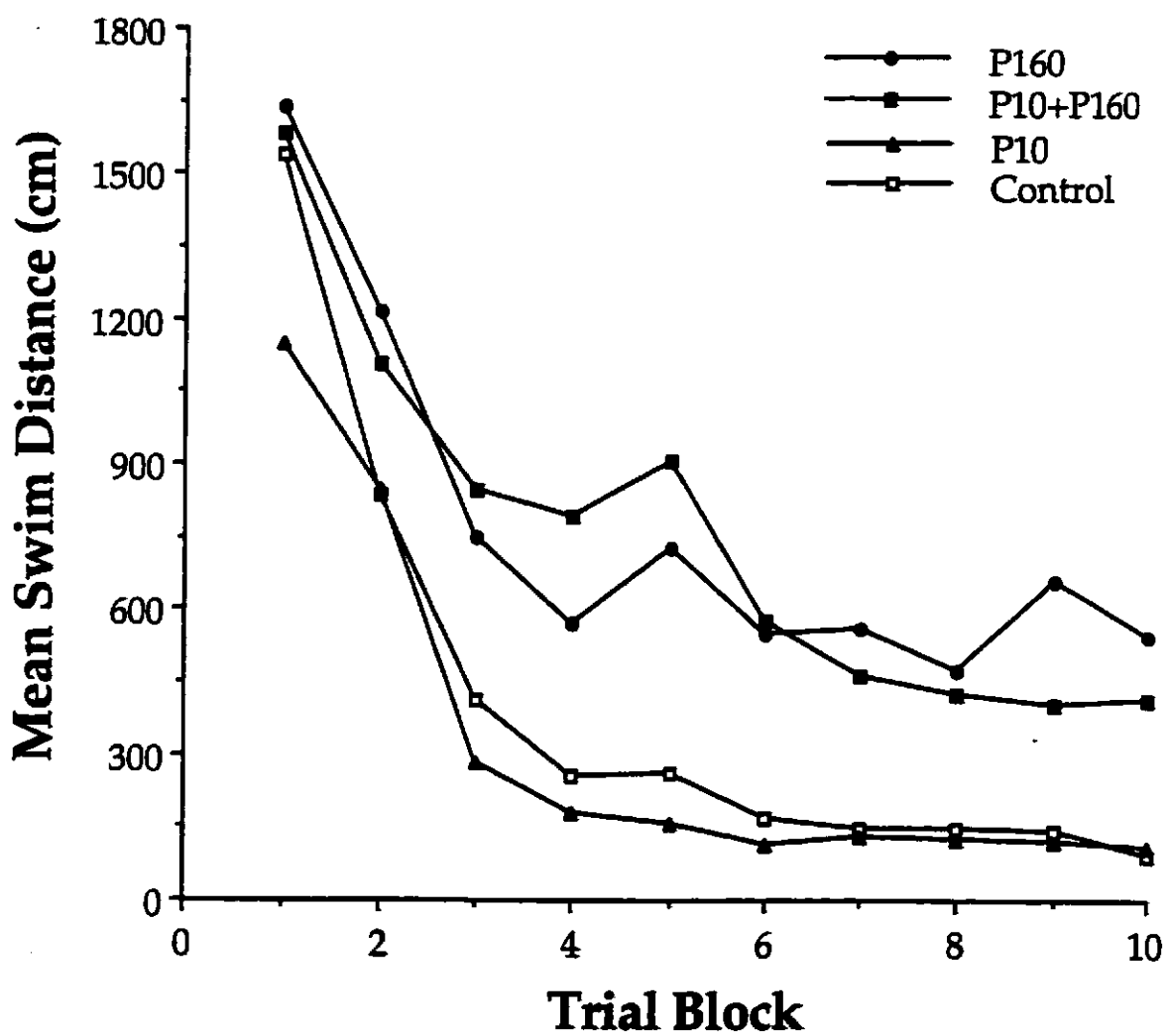


Figure 5. Swim distance per trial block

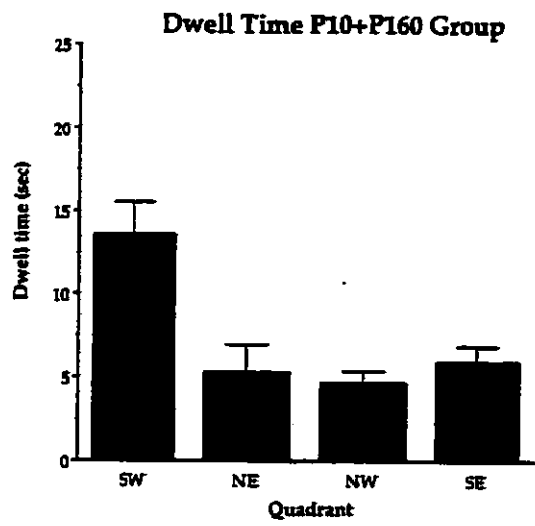
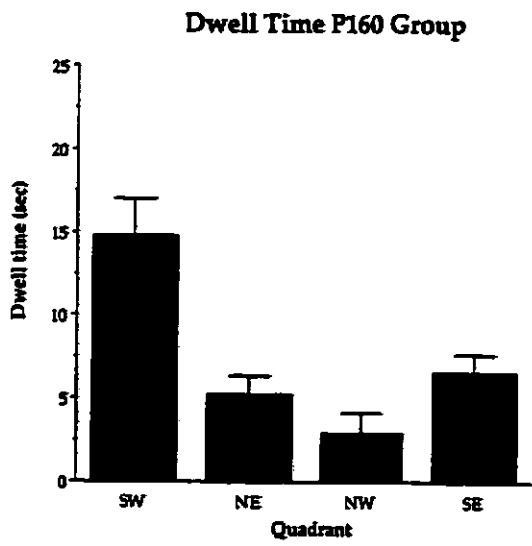
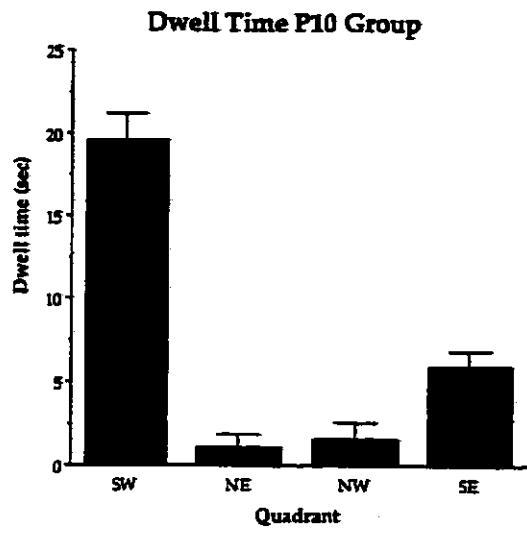
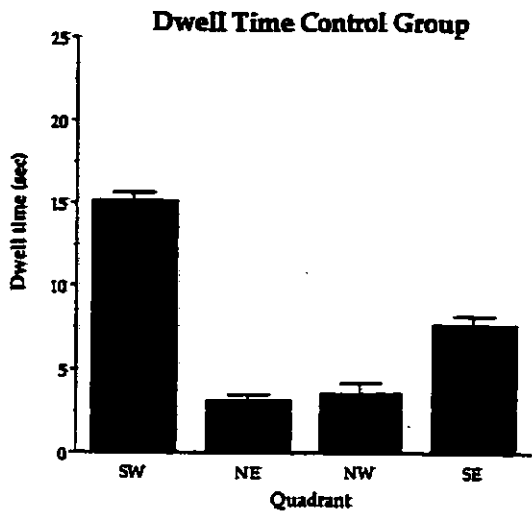


Figure 6. Dwell time per quadrant during probe trial of Morris water task

Anatomical Results:

Visual Inspection

The appearance of the lesion cavities illustrates the filling-in phenomenon in neonatal and adult rats. Figure 7 illustrates the appearance of the brains used for visual inspection. Control animals have no lesion, and thus serve as a baseline. Filled-in cavities are seen in P10 animals, whereas P160 and P10+P160 animals all have distinct cavities as a result of the adult lesions.

Brain weight

The brains of rats in all lesion groups were lighter than control brains, as might be expected. Figure 8 illustrates that brain weights in each of the lesion groups were not equivalent. It has previously been shown that neonatally lesioned animals have lighter brains in adulthood (e.g.: Kolb, 1987). The P160 groups also had lighter brains than the control, because they had tissue removed in adulthood. The P10+P160 brains were lighter than the brains in either of the other two lesion groups which was expected because these animals were subjected to a second lesion that removed even more brain tissue than the adult-only lesion.

Due to the usual sex differences in brain weight, a two-way ANOVA (Group x Sex) was performed. The ANOVA did show a significant effect of group ($F(3,32)=18.692$, $p<0.0001$), a significant effect of sex ($F(3,23)=20.903$, $p<0.0001$), and a significant interaction (Group x Sex) found between group and sex ($F(3,32)=2.949$,

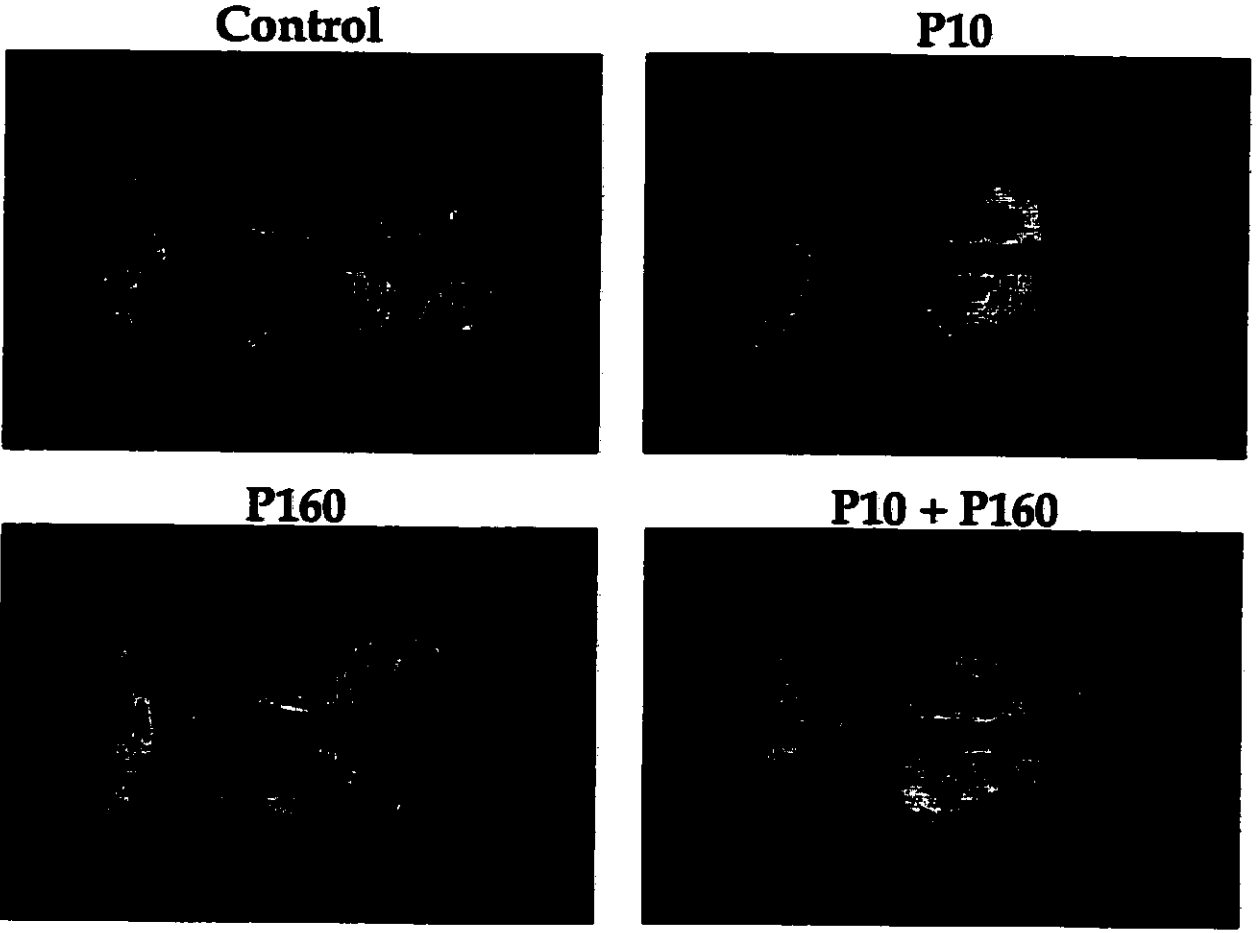


Figure 7. Pictures of representative brains

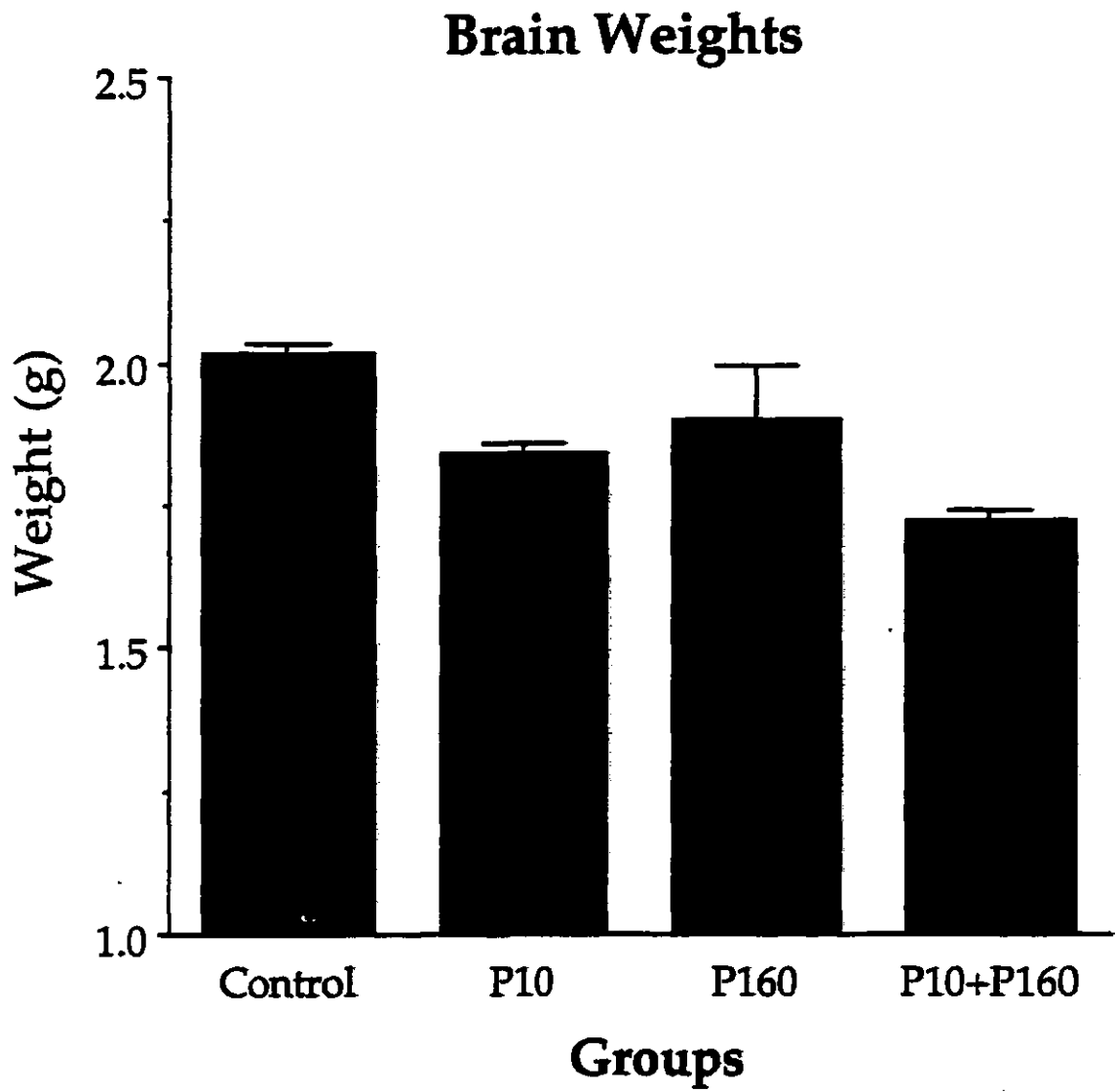


Figure 8. Brain weights by group

$p < 0.0475$). Follow-up simple ANOVA's on each sex showed an overall effect of group ($F(2,8)=4.878$, $p < 0.0412$), and post-hoc testing (Fisher's LSD) revealed that male control animals had the heaviest brains and P160 animals had the lightest. Female animals also showed a significant main effect of group on a one-way ANOVA, ($F(3,19)=14.049$, $p < 0.0001$), and post-hoc testing (Fisher's LSD) showed the control animals to have the heaviest brains, and P10+P160 group had the lightest brains.

Cortical Thickness

As can be seen in Figure 9 the lesions in this experiment caused differences in cortical thickness. Cortical thickness was measured following the procedure of Stewart (1988) and the data are consistent with the notion that neonatal lesions lead to the development of a thinner cortex than is observed in either controls or adult animals of the lesion group. A one-way ANOVA on cortical thickness showed a significant main effect ($F(3,77)=4.953$, $p < 0.0001$). Post-hoc tests (Fisher's LSD) revealed that both groups with P10 lesions had thinner cortices than the other two groups, which did not differ from each other.

Figure 10 illustrates the differences in cortical thickness at four coronal planes. A two-factor repeated measures ANOVA was used to analyze these data. The main effect of lesion group was evident ($F(3,65)=26.6$, $p < 0.0001$), as was the main effect of location of coronal plane measured. Subsequent one-way ANOVA's compared cortical thickness in both right and left hemispheres of each of the four coronal planes.

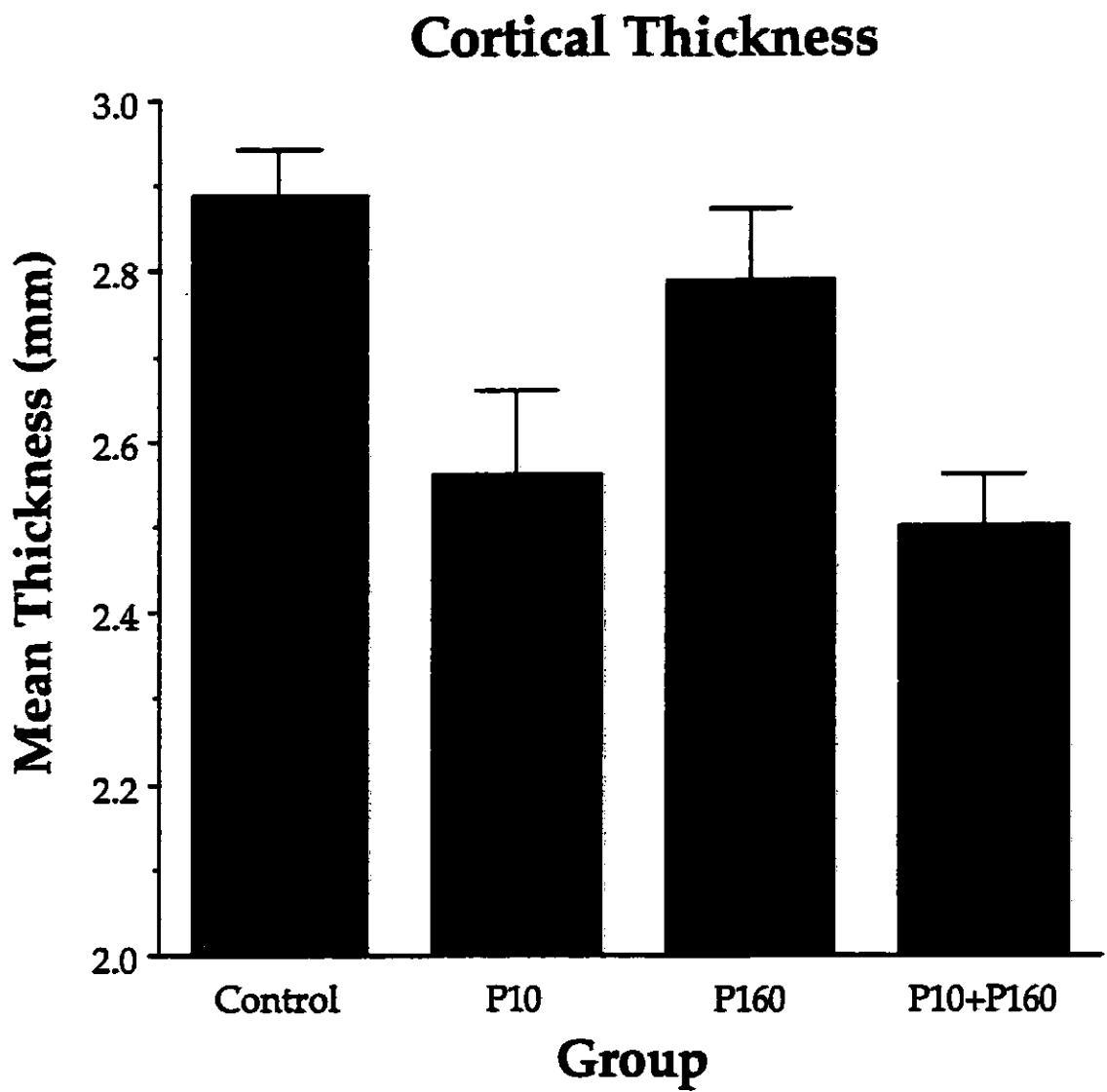


Figure 9. Mean cortical thickness by group

Planar Comparisons of Cortical Thickness

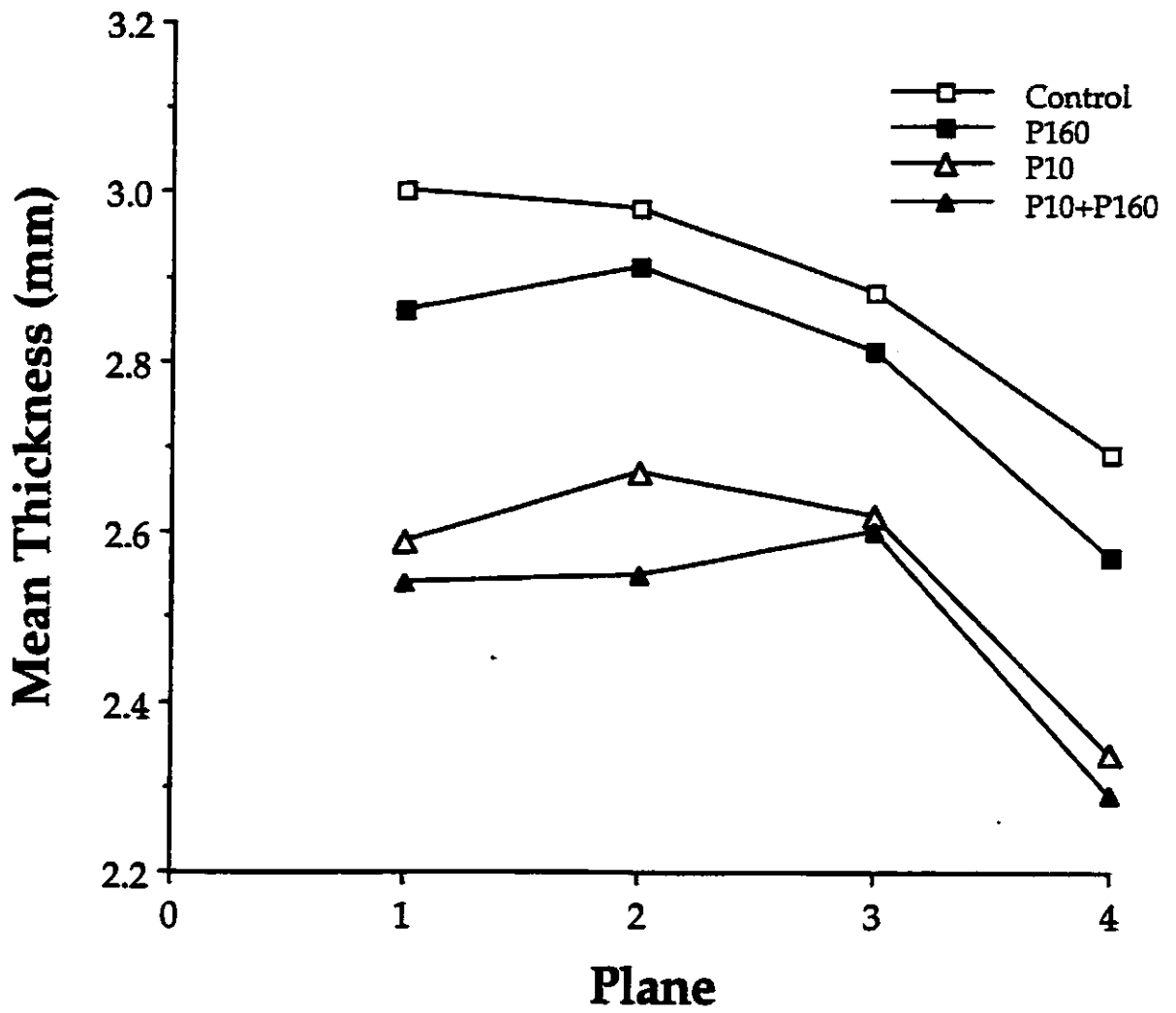


Figure 10. Mean cortical thickness per plane

Significant group effects were evident in all measurement sites (p values of 0.01 or better). The significance values of the right and left hemispheres closely resemble one another, a result that was expected due to the bilateral nature of the lesions.

Dendritic Analyses

Figures 11 and 12 summarize the total Sholl ring crossings for the apical and basilar dendrites, respectively. It is evident from these figures that there is a decrease in dendritic length of the apical dendrites of P10 lesioned animals, relative to the control and adult-operated animals. In contrast, there is an increase in basilar dendritic length of P10 operated groups.

Two-factor repeated measures ANOVA's (Group x Ring) were performed on both the apical and basilar dendrites. The comparison of apical dendrite lengths revealed significant differences in ring crossings ($F(3,48)=230.144, p<0.0001$). There were no significant differences due to group ($F(3,48)=1.4, p<0.2537$), nor was there a significant group by ring crossing interaction ($F(3,48)=1.08, p<0.3327$).

The results of the basilar dendrite analysis is markedly different from that of the apical dendrites. Significant differences were found among groups ($F(3,48)=2.862, p<0.0460$), and among ring crossings ($F(3,48)=1902.483, p<0.0001$). Also, a highly significant interaction was found in the group by dendrite length analysis ($F(3,48)=2.754, p<0.0001$).

A closer inspection of dendrites was made by analyzing the dendritic changes at

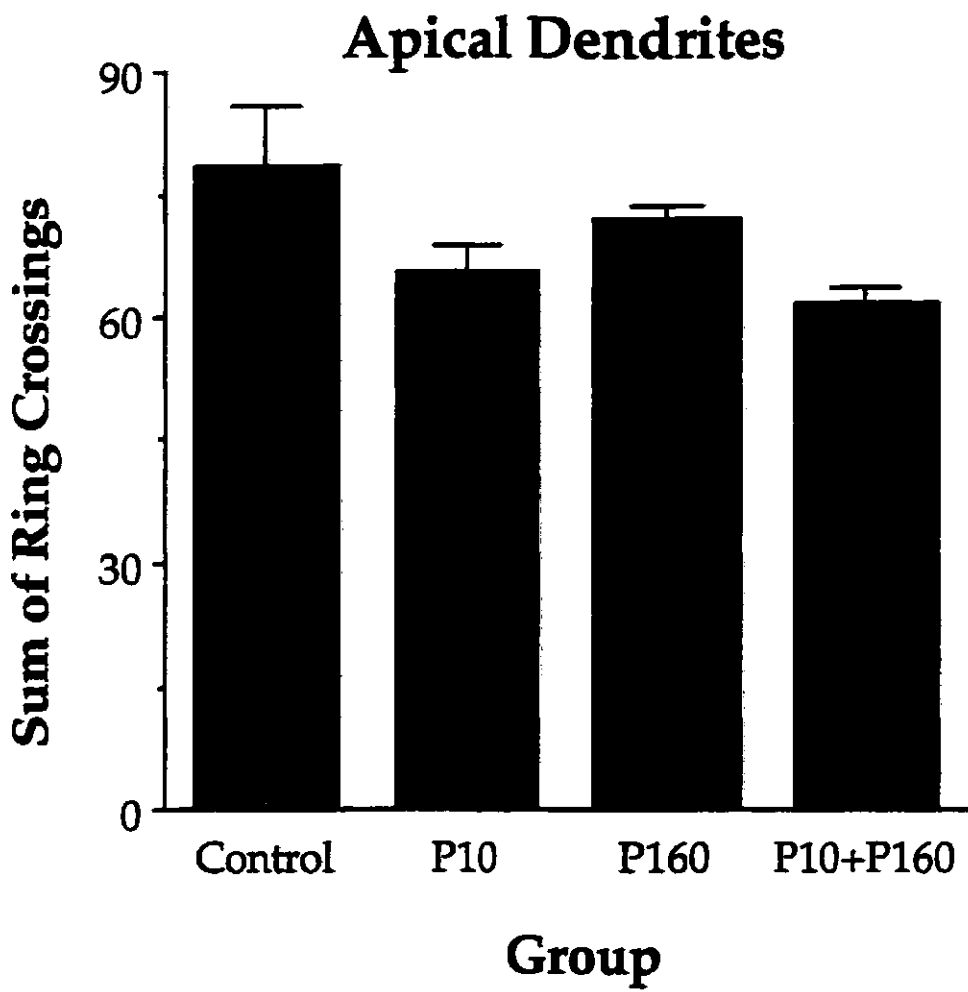


Figure 11. Total number of apical dendrite ring crossings in Par 1, layer III cells

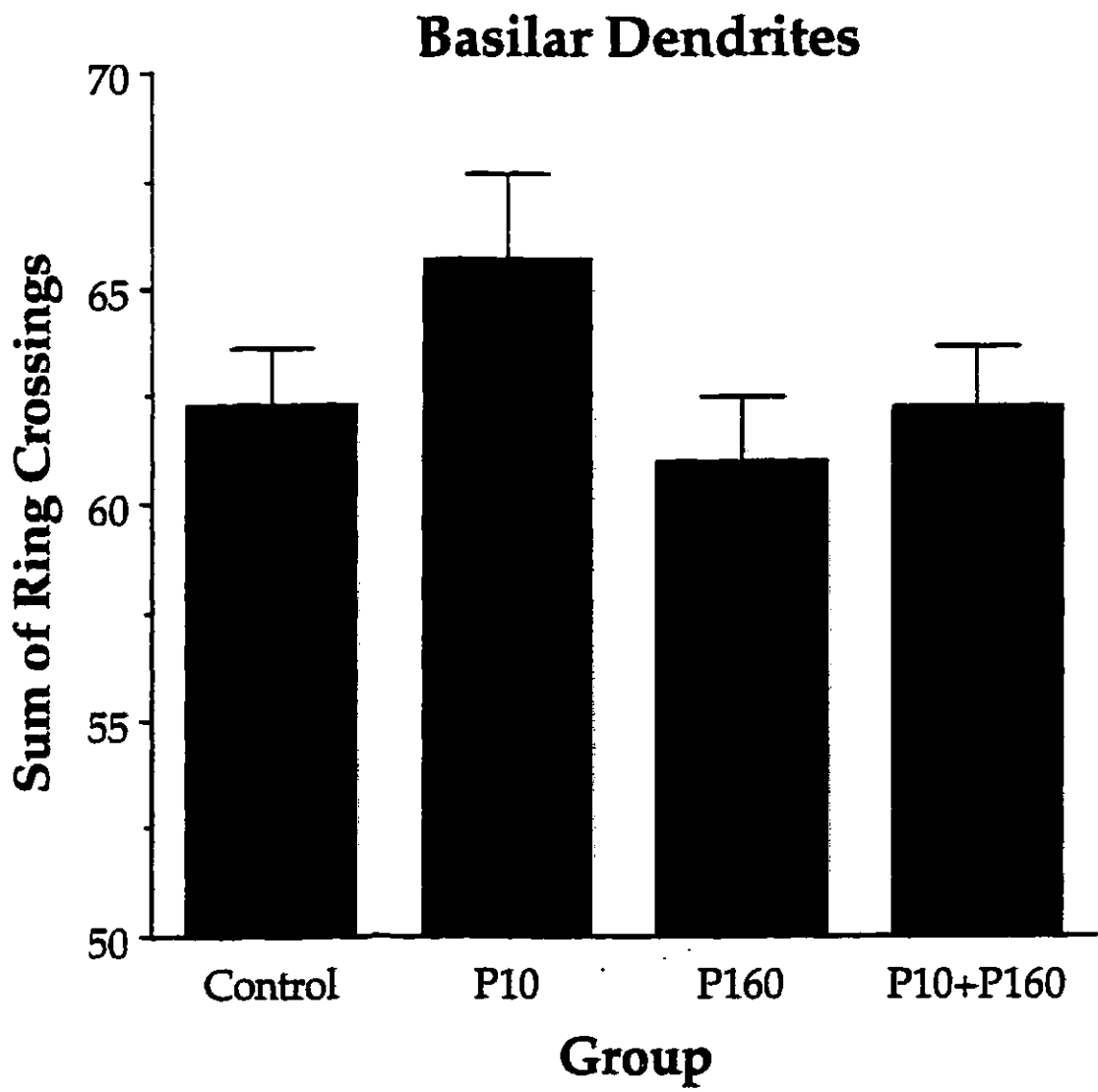


Figure 12. Total number of basilar dendrite ring crossings in Par 1, layer III cells

different segments of the dendritic fields, because it is common to find group differences in dendrite length at the distal branches of the dendrites. Figures 13 and 14 show that there were differential effects of the lesions on the dendritic arborization in the apical and basilar fields at the more distal portions of the dendrites. Thus, whereas there was an obvious drop in dendritic arborization in the distal apical dendrites of the animals with P10 lesions, there was an increase in dendritic arborization at the distal basilar dendrites. Analysis of the apical dendrites, rings 8 through 15, revealed significant group differences ($F(3,50)=7.436, p<0.0003$). The same finding was true of the distal portions of basilar dendrites, rings 6 through 10 revealed significant group differences ($F(3,50)=7.684, p<0.0003$).

Discussion

Two behavioral results suggest that rats with P10 lesions show functional recovery that is related to the regrown midline frontal tissue. First, rats with P10 frontal lesions showed nearly normal performance on the water task, a result that dramatically contrasts with performance of the rats with similar lesions in adulthood. Second, when the regrown tissue was removed in the P10+P160 animals, functional recovery was completely lost. Thus, because removal of the regrown tissue abolished recovery, it appears likely that the regrown tissue was supporting recovery of function. The anatomical results are consistent with this hypothesis. As has been shown in previous studies, rats with P10 lesions had thinner remaining cortex than do animals with adult

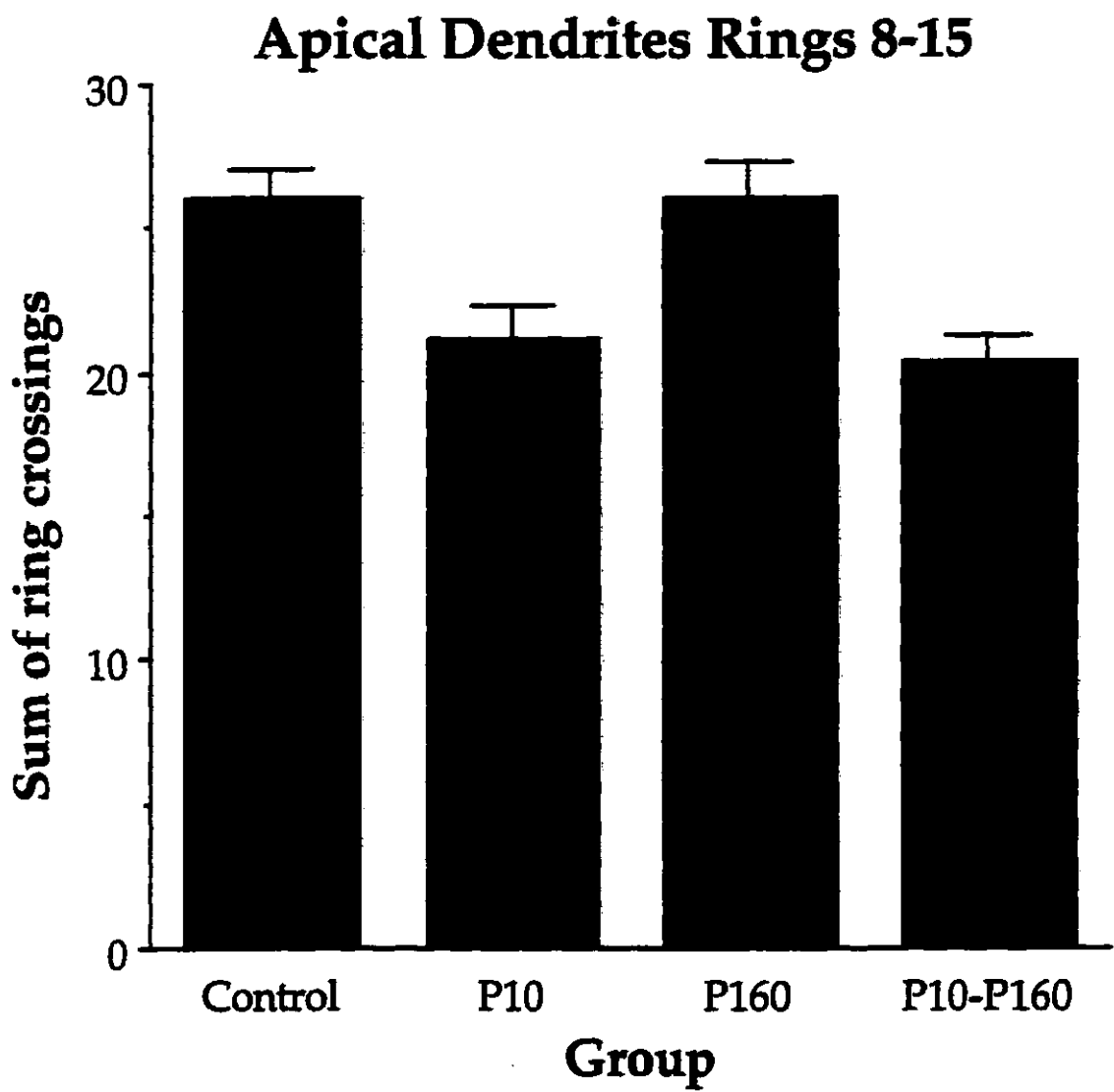


Figure 13. Sum of apical dendrite ring crossings in Par 1, layer III cells

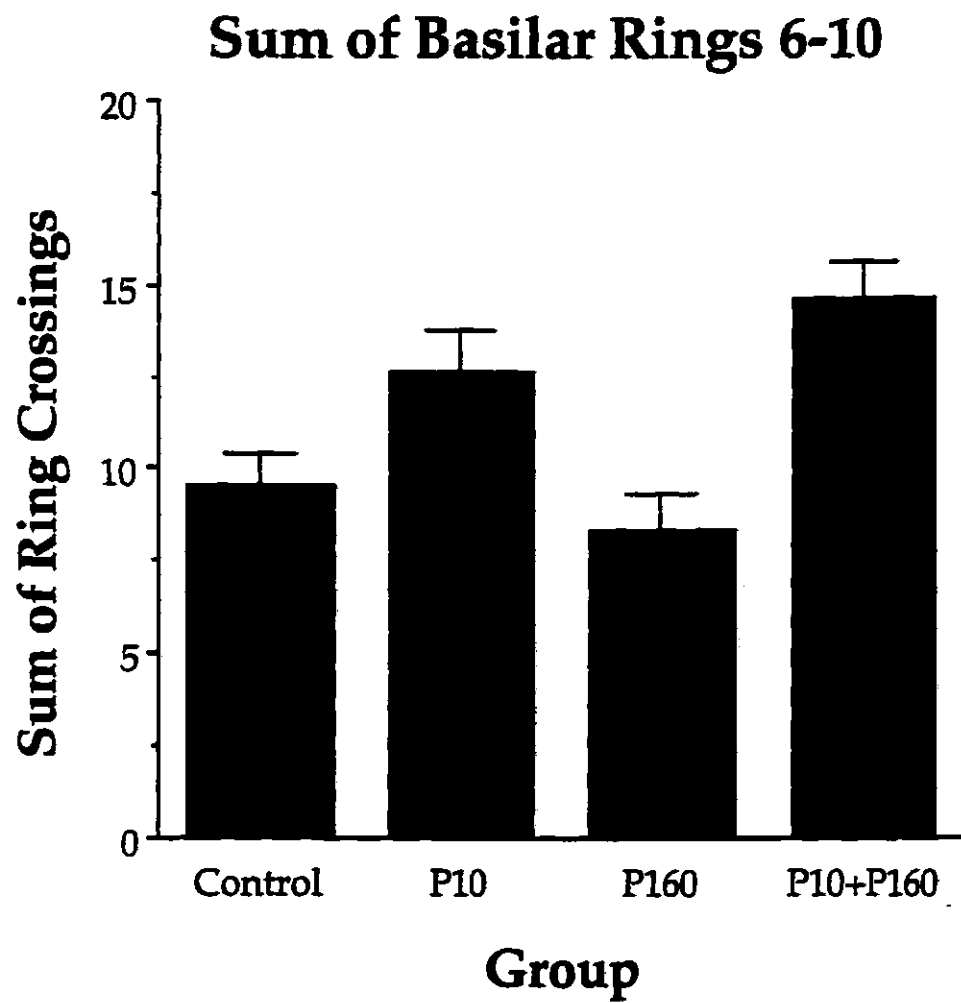


Figure 14. Total number of ring crossings of basilar dendrites of area Par1, layer III cells

lesions. Analysis of dendritic arborization showed a decrease in apical branching and only a small increase in basilar branching in rats with P10 lesions. The most parsimonious explanation for the functional recovery in the P10 animals is that the regrown midline tissue was functional.

Chapter 4. Experiment Two

The results of Experiment 1 showed that when midline frontal tissue is removed on postnatal day 10, the lesion cavity is filled-in by adulthood, and the animals show substantial behavioral recovery. It is therefore hypothesized that the filled-in tissue is at least partially responsible for the functional recovery. Although the origin of the cells in the lesion cavity is not proven beyond reasonable doubt, Kolb et al (1998) proposed that it was due to the massive upregulation of the turnover of stem cells in the subventricular zone. Thus, it was suggested that the progenitor cells were generated in the subventricular zone after the lesion and these cells were responsible for the filling-in of the lesion cavity. Because it had been shown *in vitro* that neurotrophins induce stem cells to divide and ultimately to produce neurons and glia, it was further hypothesized that the presence of endogenous neurotrophins was responsible for the initiation of neurogenesis after midline frontal injury at this age.

Two questions arise from these results. First, is it possible to generate new neurons after removal of the midline frontal cortex in adulthood? Second, is it possible to generate new neurons in the P10 injured brain twice? That is, will an infusion of neurotrophins induce neurogenesis in the adult brain that has already generated cells in infancy? Kolb et al (1998) have shown that an infusion of a cocktail of neurotrophins (EGF, NGF, and bFGF) will stimulate the subventricular zone to generate new cells that will fill in the lesion cavity. These cells do not develop neuronal phenotypes, however,

and do support significant functional recovery. The question we asked here was whether a P10 lesion might alter the stem cell activity such that a neurotrophin cocktail might stimulate the development of new cells with an appropriate neuronal phenotype, much as is seen in the spontaneous neurogeneration after a P10 lesion.

Materials and Methods

Subjects:

The study was done with 22 rats, derived from the Charles River Long-Evans strain. The distribution of the animals within the experiment was: control rats n=6 (6m), postnatal day 160 lesion rats (P160) n=6 (6m), postnatal day 160 lesion rats implanted with osmotic minipumps (P160+pump) n=6 (6m), combined postnatal day 10 and day 160 lesion rats implanted with osmotic minipumps (P10+160+pump) n=4 (4m). See Figure 15 for the experimental design.

Surgical and Behavioral Procedures

Rats were given medial frontal lesions on P10 and then again on P160, following the surgical procedures of the first experiment. Those rats that received osmotic minipumps were implanted with cannulae and pumps immediately following the second surgery. The pumps infused a cocktail of neurotrophic factors for seven days, and then were explanted. The concentration of growth factors was based upon previous *in vivo* studies and the recommended working ranges from the suppliers. Thus, we used a

Experimental Design

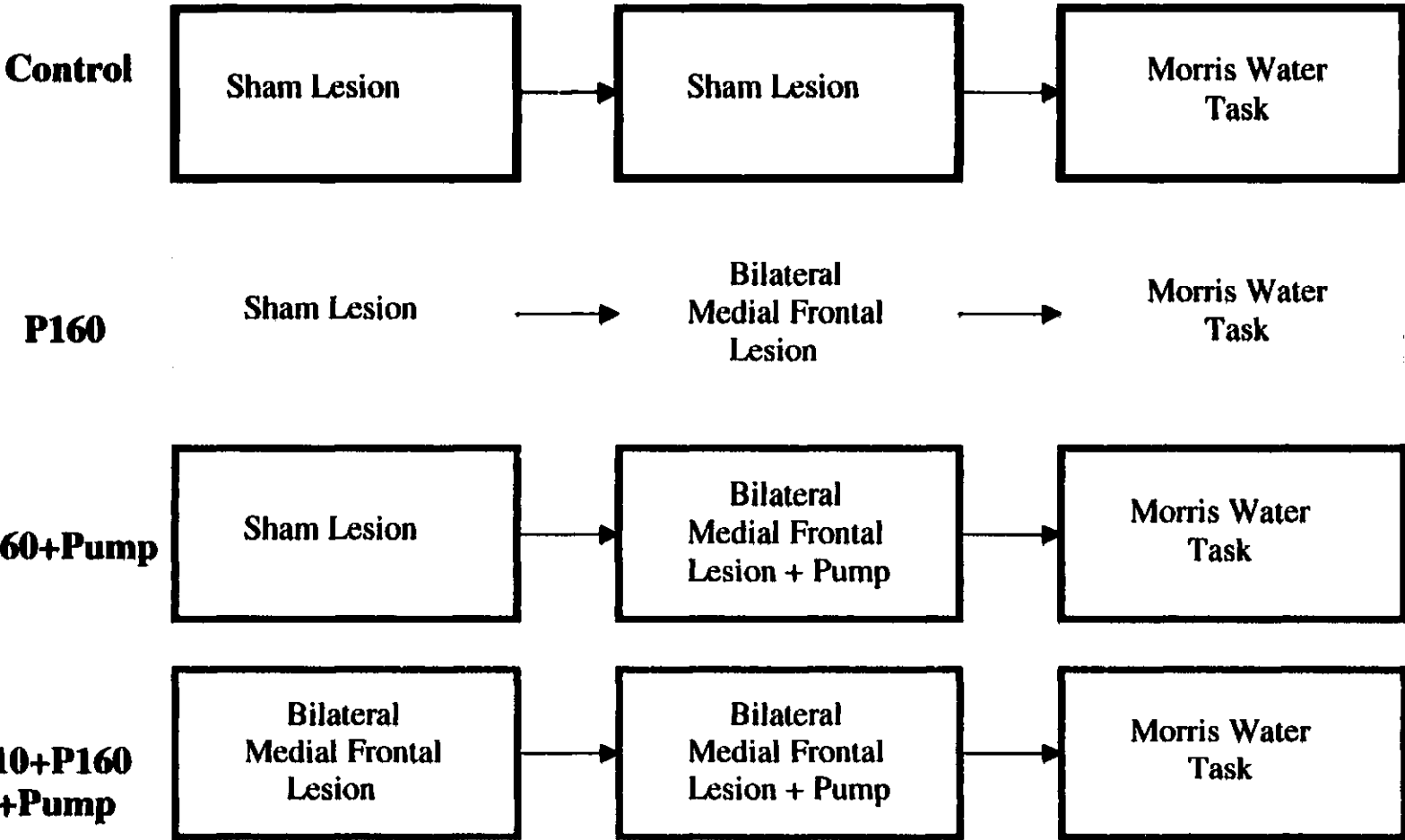


Figure 15.
48

cocktail of $10\mu\text{g}/\mu\text{L}$ EGF; $1\mu\text{g}/\mu\text{L}$ NGF; and $1\mu\text{g}/\mu\text{L}$ bFGF with a total volume of $239\pm 9\mu\text{L}$. All animals were trained in the Morris water task 60 days following the date of the second surgeries. After behavioral testing was complete, the animals were sacrificed in the same method as the first experiment, and the P10+P160+pump animals' brains were processed for Golgi-Cox staining as in Experiment 1. The remaining animals' brains were processed by perfusion with 0.9% saline followed by 4% paraformaldehyde. The data for the animals were compared to those animals in Experiment 1, as well as to animals that only received lesions on P160 and an osmotic pump containing the same neurotrophic factors.

Dendritic Analyses:

In the first experiment, Sholl analysis was performed on the apical and basilar dendrites to determine dendrite length. However, in this experiment the P160+pump brains were not suitable for Sholl analysis because they were processed for immunohistochemical staining. We will present the data for the control, P160, and P10+P160+pump groups, and for the sake of comparison, we will include the groups from the first experiment.

Results

Behavioral Results

Latencies

The overall result was that all groups with medial frontal removal on P160 (i.e.

the P160's, P160+pump, and P10+P160+pump groups), were impaired relative to controls on the Morris water task (Figures 16 and 17). A two-way ANOVA (Group x Trial Block) revealed significant differences for group ($F(3,27)=8.439, p<0.001$), for trial block ($F(3,9)=59.568, p<0.0001$), and a significant interaction between the two ($F(3,27)=5.369, p<0.0001$). Post-hoc tests (Fisher's LSD) revealed significant differences between all groups at $p<0.05$, except between P160 and P10+P160+pump groups.

Analysis of latency of trial blocks showed that all groups are virtually indistinguishable by the 10th trial block (40th trial). One-way ANOVA's on individual trial block (TB) latencies revealed significant between-group differences per trial block in TB 1, ($F(3,18)=4.91, p<0.0115$); TB 2, ($F(3,18)=14.04, p<0.0001$); TB 3, ($F(3,18)=10.68, p<0.0003$); TB 4, ($F(3,18)=4.23, p<0.0198$); TB 5, ($F(3,18)=3.74, p<0.0299$); and TB 9, ($F(3,18)=3.84, p<0.0276$).

Swim Distance

Swim distance was not analyzed in experiment two because the data were not available for all animals.

Anatomical Results

Visual Inspection

Visual inspection of the perfused brains (see Figure 18) illustrates the adverse effects of the second lesion in adulthood. Brain A is a P160 animal, and shows no

Place Task Performance

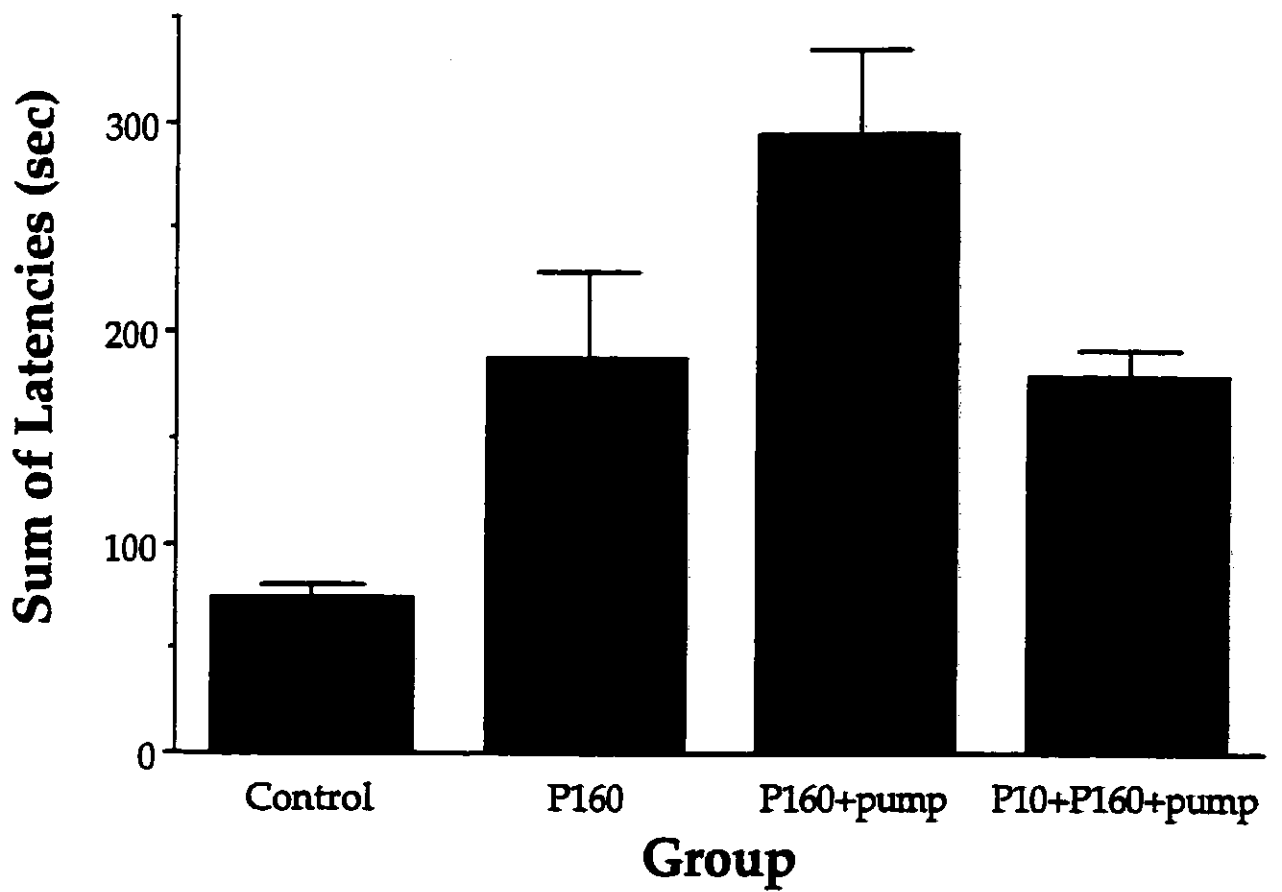


Figure 16. Total latencies of each group on Morris water task

Place Task Performance by Trial Block

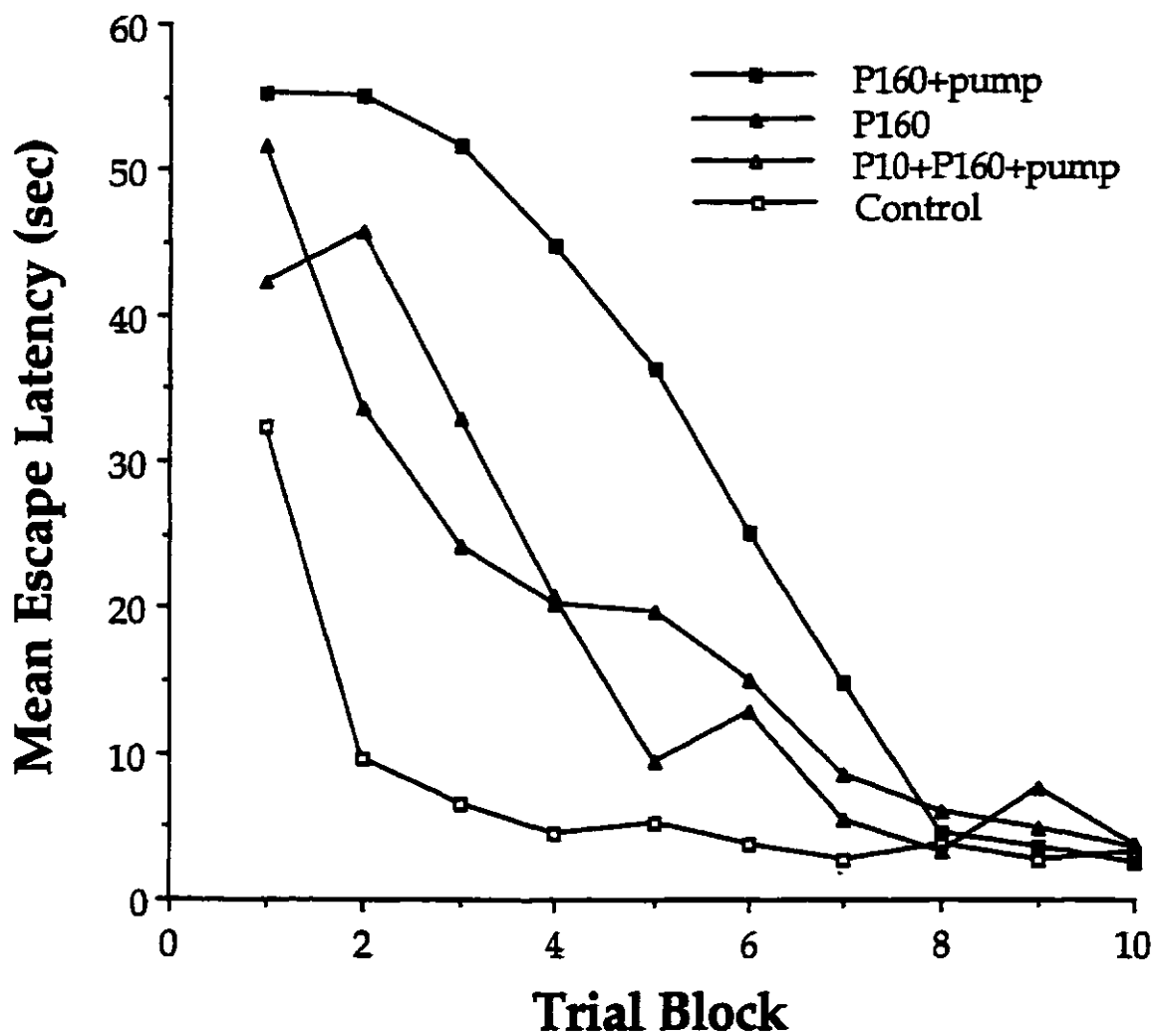


Figure 17. Mean escape latency per trial block on Morris water task

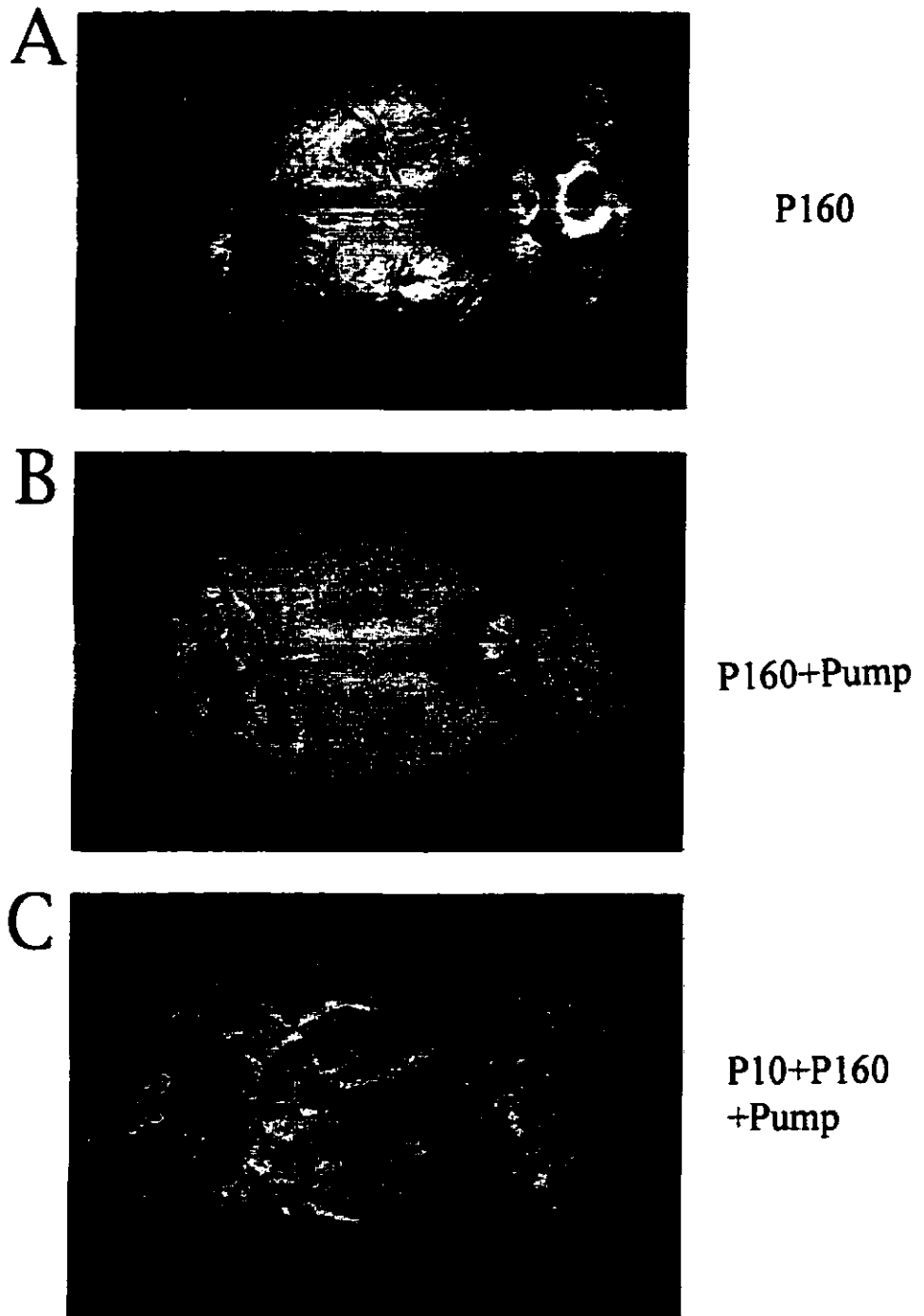


Figure 18. Pictures of representative brains

apparent filling-in of tissue in the lesion cavity, which is consistent with previous studies. Brain B, a P160+pump animal, shows some filling-in of tissue, which is to be expected when there is an infusion of NTF cocktail. Brain C is the P10+P160+pump group, and does not show the filling-in of the lesion cavity seen in brain B. Thus, it appears that the P10 lesion altered the brain's later ability to respond to the neurotrophin cocktail.

Dendritic Analysis:

The addition of the neurotrophins to animals with infant plus adult frontal lesions had little effect on the dendritic arborization (see Figure 19). Thus, the length of the apical dendrites was similar to that of the other lesion groups. A one-way ANOVA showed no overall group differences of ring crossings ($F(4,55)=1.202$, $p<0.3205$). Post-hoc analysis (Fisher's LSD) showed a significant difference only between the control and P10+P160 groups (at $p<0.05$).

Similarly, the length of the basilar dendrites was virtually identical in all three P10 groups, and was higher than control or P160 groups. A one-way ANOVA shows a significant group effect ($F(4,55)=2.596$, $p<0.0462$). Post-hoc testing (Fisher's LSD) indicates statistically significant differences between P10 and P160, P10+P160 and P160, and P160 and P10+P160+pump groups (at $p<0.05$).

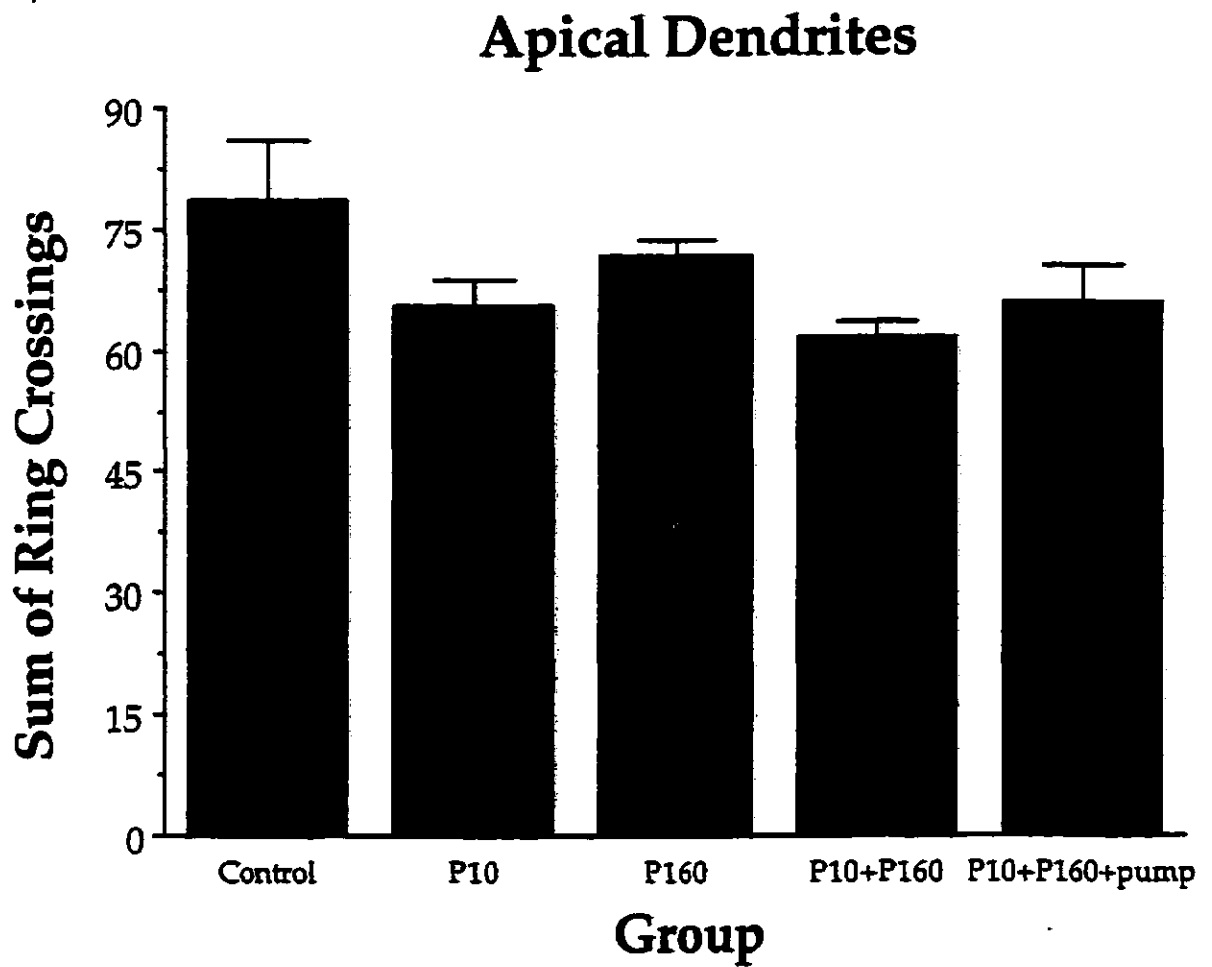


Figure 19. Total number of apical dendrite ring crossings of Par 1, layer III cells

Discussion

There were two main questions asked at the beginning of this experiment: the first was whether it was possible to generate new neurons after medial-frontal lesions in adulthood, and the second question asked whether it is possible to generate new neurons in the P10 injured brain in adulthood. Based on the evidence gathered in this experiment, it appears that generation of new neurons does not occur spontaneously in the adult injured brain, as it does in the P10 brain. However, the addition of neurotrophins to the adult injured brain promotes filling-in of the lesion cavity, and may influence the architecture of the rest of the brain. Thus, the NTF's might be expected to improve performance because the rest of the brain was changed as well.

Results of the first experiment suggest that spontaneous regeneration of neurons occurs in P10 lesioned animals. We suggest that the mechanism of recovery following neonatal lesions can be attributed to migration of stem cells from the sub-ventricular zone of the brain to the lesion cavity, contributing to the functional and anatomical recovery of the animals. However, when infant and adult lesions are combined, there is not full recovery as seen in P10 lesion-only animals. We therefore conclude that stem cells are able to repair the frontal cortex only once and the effects of a second lesion are not ameliorated even by the addition of NTF's.

Chapter 5. General Discussion

There are four principal findings of these studies.

1. Animals with P10 frontal lesions showed functional recovery that was correlated with a filling in of the lesion cavity and hypertrophy of basilar dendrites in layer III pyramidal cells.
2. Removal of the filled-in tissue produced a severe behavioral deficit but did not alter the dendritic hypertrophy.
3. The infusion of neurotrophic factors after adult frontal lesions resulted in the filling of the lesion cavity with undifferentiated cells. There was no behavioral improvement in the water task.
4. The infusion of neurotrophic factors after P10+P160 frontal lesions did not stimulate the generation of cells, suggesting that the generation of new cells after an early frontal injury alters the later activity of stem cells in the brain.

I will consider each of these findings separately.

Effects of P10 lesions

Kolb and his colleagues have shown in a series of studies that removal of the medial frontal cortex at 7-10 days of age is associated with significant functional recovery. In contrast, similar frontal removals in the first five or fewer days postnatally is associated with a dismal functional outcome (Kolb et al., 1996a; Kolb et al., 1996c). In their early experiments, Kolb et al found that large removals of frontal cortex, including medial

frontal cortex and anterior motor areas, was associated with dendritic hypertrophy and increased spine density when the lesions were made at seven to ten days of age, and dendritic atrophy and decreased spine density when lesions were sustained at one to five days of age (e.g.: Kolb, 1995). Later studies showed that when the lesions were restricted to the midline frontal cortex, there was a second, and rather unexpected, filling-in of the lesion cavity when the lesions were made earlier or later (e.g.: Kolb et al., 1996c; Kolb et al., 1997d). The current two experiments confirm this observation. The filling-in of the lesion cavity was evident by visual inspection, and the animals' behavioral performance was equal to that of control animals. The dendritic expansion was restricted to the basilar dendrites and did not involve the apical dendrites in the current study, possibly because the lesions were more restricted in size than in the original Kolb and Gibb studies. Nonetheless, the functional recovery observed in these studies is correlated with two possible compensatory events: the growth of new tissue on the medial frontal area, and the dendritic changes in adjacent cortex.

Removal of filling

One of the key findings of these studies is that when the filled-in area was removed, functional recovery was lost and yet dendritic changes persisted. Thus, although the actual neural basis of the observed recovery cannot be proven definitively, it appears likely that the principal basis for the functional recovery is the regrown cortical tissue. If the tissue that regrew following the P10 lesions had not been functional, the animals should not have suffered any behavioral effect as a result of the removal, but

clearly they did. The most parsimonious explanation for these results is that the regrown tissue was functional in P10 animals, and its removal produced a behavioral deficit consistent with adult-lesioned animals. It should be noted that it is impossible to determine whether the new cells have established connections that are precisely the same as normal cells, without the use of retrograde and anterograde labels.

One difficulty with this explanation is that the animals with the second lesion to remove the filled-in tissue were actually more severely impaired at the water task than adults with similar removals. One explanation for this finding is that the rats with removal of the filled-in tissue have larger lesions than adults, due to the surgical procedure. Fantie and Kolb (1990) have shown that adult rats with medial frontal lesions are more impaired at the water task when significant amounts of adjacent motor cortex are also removed. Thus, if the second lesion removed filled-in tissue as well as adjacent motor cortex, we can predict that the reason for the severe behavioral deficit is lesion size. Unfortunately, owing to the experimental procedure of suction aspiration of the filled-in area, it is not possible to examine this question directly. There are two reasons to doubt this explanation, however. First, the behavioral deficit observed in the current animals was much more severe than in the large lesions animals in the Fantie and Kolb study. Second, as in past experiments, the animals with P10 lesions have a thinner cortical mantle in adulthood than in adult frontal operates. It seems likely that the remaining cortex is less efficient at cognitive operations than normal tissue and this could certainly compromise water task performance.

Another piece of evidence strengthening the idea that the filled-in tissue was

supporting functional recovery comes from a parallel set of experiments in which the regrowth of the medial frontal area was blocked with embryonic injections of the mitotic marker, bromodeoxyuridine (BrdU) (Kolb, Martens, Gibb, Coles, & van der Kooy, 1999a; Kolb, Pedersen, Ballermann, Gibb, & Whishaw, 1999b). In the course of studying the genesis of new neurons in animals with medial frontal lesions, Kolb et al discovered that injections of BrdU on embryonic day 13 prevents the later filling-in of the lesion cavity after a P10 frontal lesion. The absence of cortical regrowth is associated with behavioral deficits in the water task that are as severe as those observed in the animals with filled-in tissue removed in the current study. In other words, removing the medial frontal filling has behavioral sequelae that are similar to those when the filling is prevented.

Based on the combination of data from the present experiment and the BrdU experiments blocking the filling of the lesion cavity, it is not likely that the explanation for the recovery of animals in the present study is attributable solely to compensation. If the recovery were compensatory, the animals in the BrdU studies that did not display filling-in of the lesion cavity would have shown at least partial recovery.

Neurotrophic factors and recovery

It has been shown that various neurotrophic factors influence functional recovery after cortical lesions (e.g.: Kolb et al., 1997b; Rowntree & Kolb, 1997). In the current study a cocktail of NGF, EGF, and bFGF was infused into the ventricle of rats with cortical lesions in adulthood or after the combined infant-adult lesions. The addition of

the neurotrophic factors following the adult lesions produced partial filling-in of the lesion cavity, but did not restore function of the cells when measured by swim latency. The lesion cavity was irregularly and incompletely filled in, and the cells did not differentiate into neurons, but rather remained undifferentiated cells that stained poorly with the Golgi procedures. There apparently was no cell generation in response to the neurotrophic cocktail in the P10+P160 animals, although the Golgi procedure is not optimal for identifying new cells. Curiously, although previous studies have found an increase in dendritic length in the pyramidal cells in the midline cortex of animals treated with NGF, there were no changes in parietal cells in the NGF-treated cells in the current study. It may be that the pyramidal cells in the parietal cortex are not responsive to NGF or it may be that the cocktail of NGF, EGF, and bFGF has different actions on neuropil than NGF alone. Nevertheless, the animals administered the neurotrophic cocktail and the P10+P160 lesions did show improvement in the water task performance. The simplest explanation is that the neurotrophic factors were modifying the brain in other regions or in ways that we did not assess in the current study. For example, there may have been dendritic changes in regions such as posterior parietal cortex, or there may have been glial or vascular changes in any number of cerebral regions. Unfortunately, the camera lucida procedure to quantify cellular changes is extremely time-consuming, and examination of dendrites of all areas of the brain is impractical.

The explanations for recovery differ in experiments one and two, and I propose that the difference is due to the addition of neurotrophins in experiment two. The overall conclusion for experiment one is that rats with neonatal lesions display a complete

functional recovery attributable to the filled-in tissue. The conclusion for experiment two, however, is that some other mechanism is responsible for recovery, as very little or no filling is seen. It is not possible to unequivocally conclude that addition of neurotrophins in experiment two is the explanation for functional improvement, as neither posterior parietal cortex, glial, nor vascular changes were examined in the experiments.

Stem cell response

The cause of the cavity filling in the P10 animals has been proposed to be the result of the generation of new progenitor cells by the subventricular zone. These progenitor cells are believed to migrate to the lesion cavity and then to reform at least part of the medial frontal area (e.g. Kolb et al., 1998b). Recently, Kolb et al (1999) have found that both early frontal lesions and BrdU treatment alter the activity of the subventricular stem cells in vitro. The subventricular zone of mice given frontal lesions on postnatal day seven were harvested in adulthood and cultured with either EGF or bFGF. The subventricular zone of the mice produced less than 50% as many new stem cells in vitro than in the subventricular zone of untreated control mice. When the newly-generated stem cells were removed and passaged a second time in the trophic factors, the cells from the lesion animals produced virtually no new cells whereas there was still massive proliferation in the cells from control animals. It thus appears that the early lesions interfere with later stem cell activity. We can speculate that the regeneration of the midline frontal tissue after the frontal lesions in infancy taxes the stem cells and

renders them unable to respond as vigorously later. If so, this would provide an explanation for the failure to find a response to the neurotrophic factors in the P10+P160 animals in the current study, though there was a response by adult-lesion animals. It appears that the stem cells generated new neurons after the P10 lesions and are unable to respond to the neurotrophic factors in adulthood. In contrast, the stem cells in the adult animals respond vigorously to the neurotrophic factors, leading to the filling of the cavity with new, albeit abnormal, tissue.

Implications for future studies

The primary goal of the current studies was to demonstrate that the tissue that fills the lesion cavity after a P10 lesion is functional. The answer to this question was “yes”. The implication of this result is that the brain is capable of generating new functional cortex after that age that neocortical neurogenesis is usually thought to be complete. Human patients having suffered organic or traumatic brain damage could benefit from cortical neurogenesis, as damaged cells could be replaced and normal connections established by the new cells. A practical example of a human benefiting from cortical neurogenesis is an individual who undergoes brain surgery to remove pathological tissue that is inducing seizures. Without cortical neurogenesis, the person must accept the loss of function resulting from the removed tissue. However, if it is the case that adult cortical neurogenesis takes place, the person can be hopeful for at least partial recovery. Future studies will need to focus on stimulating similar regeneration in animals with cortical injury in adulthood, as well as neonates and juveniles. A secondary

goal of these experiments was to determine if it was possible to stimulate the brain to generate new cells more than once. The answer to this question appears to be "no"; the generation of new cells appears to be possible only once. The clearest implication of this finding is that early brain damage may fundamentally alter the brain's response to later injury. Future studies may want to determine if this result is true generally of early brain injury, or if it is specific to damage to the medial frontal cortex. In addition, because it is now known that neurogenesis continues in the olfactory bulb and dentate gyrus of the hippocampus throughout adulthood, it should be determined whether there is an effect of early cerebral injuries in the normal generation of olfactory or hippocampal neurons.

References

- Altman, J. (1963). Autoradiographic investigation of cell proliferation in the brains of rats and cats. *Anatomical Records*, *145*, 573-591.
- Bach-y-Rita, P., & Bach-y-Rita, E. (1990). Biological and psychosocial factors in recovery from brain damage in humans. *Canadian Journal of Psychology*, *44*(2), 148-165.
- Barde, Y.-A. (1989). Trophic factors and neuronal performance. *Neuron*, *2*, 1525-1534.
- Bayer, S. A. (1983). Tritiated thymidine-radiographic studies of neurogenesis in the rat olfactory bulb. *Experimental Brain Research*, *50*, 329-340.
- Bayer, S. A. (1985). Neuron Production in the hippocampus and olfactory bulb of the adult rat brain: addition or replacement? *Annals of the New York Academy of Sciences*, *457*, 163-172.
- Bayer, S. A., Yackel, J. W., & Puri, P. S. (1982). Neurons in the rat dentate gyrus granular layer substantially increase during juvenile and adult life. *Science*, *216*, 890-892.
- Birse, S. C., Leonard, R. B., & Coggeshall, R. E. (1980). Neuronal increase in various areas of the nervous system of the guppy, *Lebistes*. *Journal of Comparative Neurology*, *194*, 291-301.
- Bjorklund, A., & Stenevi, U. (1972). Nerve growth factor: stimulation of regenerative growth of central noradrenergic neurons. *Science*, *175*, 1251-1253.
- Brown, M. C., Hopkins, W. G., & Keynes, R. J. (1991). *Essentials of Neural*

Development: Cambridge University Press.

Chen, K. S., Masliah, E., Mallory, M., Terry, R. D., & Gage, F. (1995). Synaptic loss in cognitively impaired aged rats is ameliorated by chronic human NGF infusion. *Neuroscience*, 68, 19-27.

Cohen-Corey, S., Dreyfus, C. F., & Black, I. B. (1991). NGF and excitatory neurotransmitters regulate survival and morphogenesis of cultured cerebellar Purkinje cells. *Journal of Neuroscience*, 11, 462-471.

Conel, J. L. (1939). *Postnatal development of the human cerebral cortex: The cortex of the newborn*. (Vol. 1). Cambridge, MA: Harvard University Press.

Conel, J. L. (1941). *Postnatal development of the one-month old infant*. (Vol. 2). Cambridge, MA: Harvard University Press.

Conel, J. L. (1947). *Postnatal development of the human cerebral cortex*. (Vol. 3). Cambridge, MA: Harvard University Press.

Conel, J. L. (1951). *Postnatal development of the human cerebral cortex: The cortex of the six-month infant*. (Vol. 4). Cambridge, MA: Harvard University Press.

Conel, J. L. (1955). *Postnatal Development of the human cerebral cortex: The cortex of the fifteen-month infant*. (Vol. 5). Cambridge, MA: Harvard University Press.

Conel, J. L. (1959). *Postnatal development of the human cerebral cortex: The cortex of the twenty-four month infant*. (Vol. 6). Cambridge, MA: Harvard University Press.

Conel, J. L. (1963). *Postnatal development of the human cerebral cortex: The cortex of a forty-eight month infant*. (Vol. 7). Cambridge, MA: Harvard University Press.

Conel, J. L. (1967). *Postnatal development of the human cerebral cortex: The cortex of*

- the seventy-two month old infant.* (Vol. 8). Cambridge, Ma: Harvard University Press.
- Cuello, A. C., Maysinger, D., & L.Garofalo. (1992). Trophic factor effects on cholinergic innervation in the cerebral cortex of the adult rat brain. *Molecular Neurobiology*, 6, 451-461.
- Eccleston, P. A., & Silberberg, D. H. (1985). Fibroblast growth factor is a mitogen for oligodendrocytes in vitro. *Developmental Brain Research*, 21, 315-318.
- Field, T., Schanberg, S. M., Scaffidi, F., Bauer, C. R., Vega-Lahr, N., Garcia, R., Nystrom, J., & Kuhn, C. M. (1986). Tactile/kinesthetic stimulation on preterm neonates. *Pediatrics*, 77, 654-658.
- Finger, S., & Wolf, C. (1988). The "Kennard Effect" before Kennard. *Archives of Neurology*, 45, 1136-1142.
- Folkman, J., & Klagsbrun, M. (1987). Angiogenic Factors. *Science*, 235, 442-447.
- Gage, F. H., Tuszynski, M., Yoshida, K., & Higgins, G. (Eds.). (1991). *Nerve growth factor expression and function in the CNS*. Berlin: Springer-Verlag.
- Garcia-Verdugo, J. M., Llahi, S., Ferrer, L., & Lopez-Garcia, C. (1989). Postnatal neurogenesis in the olfactory bulbs of a lizard. A tritiated thymidine autoradiographic study. *Neuroscience Letters*, 98, 247-252.
- Garofalo, L., & Cuello, A. C. (1992). Nerve growth factor induced synaptogenesis and hypertrophy of cortical cholinergic terminals. *Proceeding of the National Academy of Science*, 89, 2639-2643.
- Gibb, R., & Kolb, B. (1998). A method for vibratome sectioning of Golgi-Cox stained

- whole rat brain. *Journal of Neuroscience Methods*, 79, 1-4.
- Goldman, S. A., & Nottebohm, F. (1985). Neuronal production, migration, and differentiation in a vocal control nucleus of the adult female canary brain. *Proceedings of the National Academy of Science*, 80, 2390-2394.
- Gomez-Pinilla, F., & Cotman, C. W. (1992). Transient lesion-induced increase of basic fibroblast growth factor and its receptor in layer VIb (subplate cells) of the adult rat cerebral cortex. *Neuroscience*, 49(4), 771-780.
- Gospodarowicz, D., Neufeld, G., & Schwiegerer, L. (1986). Fibroblast growth factor. *Molecular and Cellular Endocrinology*, 46, 187-204.
- Gould, E., Cameron, H. A., Daniels, D. C., Wooley, C. S., & McEwan, B. S. (1992). Adrenal hormones suppress cell division in the adult rat dentate gyrus. *Journal of Neuroscience*, *In Press*.
- Gould, E., Reeves, A. J., & Gross, C. G. (1999). Neurogenesis in the neocortex of adult primates. *In press*.
- Hebb, D. O. (1949). *The organization of behavior*. New York: John Wiley and Sons Inc.
- Hefti, F., Dravid, A., & Hartikka, J. (1984). Chronic intraventricular injections of nerve growth factor elevate hippocampal choline acetyltransferase activity in adults with partial septo-hippocampal lesions. *Brain Research*, 293, 305-309.
- Henderson, C. E. (1996). Role of neurotrophic factors in neuronal development. *Current Opinion in Neurobiology*, 6, 64-70.
- Kaplan, M., & Bell, D. (1984). Mitotic neuroblasts in the 9-day-old and 11-month-old rodent hippocampus. *Journal of Neuroscience*, 4, 1429-1441.

- Kennard, M. (1936). Age and other factors in motor recovery from precentral lesions in monkeys. *American Journal of Physiology*, *115*, 138-146.
- Kennard, M. (1942). Cortical reorganization of motor function. *Archives of Neurology and Psychiatry*, *48*, 227-240.
- Kolb, B. (1987). Recovery from early brain 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. (1995). *Brain Plasticity and Behavior*. Mahwah: Laurence Erlbaum Associates.
- Kolb, B. (1999). Towards an ecology of cortical organization: Experience and the changing brain. In J. Grafman & Y. Christen (Eds.), *Neuronal plasticity: Building a bridge from the laboratory to the clinic*. New York: Springer-Verlag.
- Kolb, B., Cioe, J., & Muirhead, D. (1998a). Cerebral morphology and functional sparing after prenatal frontal cortical lesions in rats. *Behavioral Brain Research*, *91*(1-2), 143-155.
- Kolb, B., Cote, S., Ribeiro-da-Silva, A., & Cuello, A. C. (1997a). Nerve growth factor treatment prevents dendritic atrophy and promotes recovery of function after cortical injury. *Neuroscience*, *76*, 1139-1151.
- Kolb, B., Cote, S., Ribeiro-da-Silva, A., & Cuello, C. (1997b). Nerve growth factor treatment prevents dendritic atrophy and promotes recovery of function after cortical injury. *Behavioral Brain Research*, *91*, 143-145.
- Kolb, B., Forgie, M., Gibb, R., Gorny, G., & Rowntree, S. (1996a). Age, experience, and the changing brain. *Neuroscience*.

- Kolb, B., Gibb, R., Gorny, G., & Whishaw, I. Q. (1998b). Possible regeneration of rat medial frontal cortex following neonatal frontal lesions. *Behavioral Brain Research, 91*, 127-141.
- Kolb, B., Gibb, R., & van der Kooy, D. (1994). Neonatal frontal cortical lesions in rats alter structural connectivity. *Brain Research, 645*, 85-97.
- Kolb, B., Gorny, G., S, S. C., Ribeiro-Da-Silva, A., & Cuello, C. (1997c). Nerve growth factor stimulates growth of pyramidal neurons in young adult rats. *Brain Research, 751*, 289-294.
- Kolb, B., Ladowski, R., Gibb, R., & Gorny, G. (1996b). Does dendritic growth underly recovery from neonatal occipital lesions in rats. *Behavioral Brain Research, 77*, 125-133.
- Kolb, B., Martens, D. J., Gibb, R., Coles, B., & Kooy, D. v. d. (1999a). Proliferation of neural stem cells invitro and in vivo is reduced by infant frontal cortex lesions or prenatal BrdU. *Society for Neuroscience Abstracts, 25*.
- Kolb, B., Pedersen, B., Ballermann, M., Gibb, R., & Whishaw, I. Q. (1999b). Embryonic and postnatal injections of BrdU produce age-dependent morphological and behavioral abnormalities. *Journal of Neuroscience, 19(6)*, 2337-2346.
- Kolb, B., Petrie, B., & Cioe, J. (1996c). Recovery from early cortical damage in rats. VII. Comparison of the behavioral and anatomical effects of medial prefrontal lesions at different ages of neural maturation. *Behavioral Brain Research, 79*, 1-14.
- Kolb, B., Stewart, J., & Sutherland, R. J. (1997d). Recovery of function is associated with increased spine density in cortical pyramidal cells after frontal lesions and/or

- noradrenaline depletion in neonatal rats. *Behavioral Brain Research*, 89(1-2), 61-70.
- Kolb, B., & Whishaw, I. Q. (1981). Neonatal frontal lesions in the rat: sparing of learned but not species-typical behavior in the presence of reduced brain weight and cortical thickness. *Journal of Comparative and Physiological Psychology*, 95, 863-879.
- Kolb, B., & Whishaw, I. Q. (1998). Brain plasticity and behavior. *Annual Review of Psychology*, 49, 43-64.
- Kolb, B., & Whishaw, I. Q. (In press). Reorganization of function after cortical lesions in rodents. In H. S. Levin & J. Grafman (Eds.), *Neuroplasticity and recovery of function after brain injury*. New York: Oxford University Press.
- Kolb, B., Zaborowski, J., & Whishaw, I. Q. (1989). Recovery from early cortical damage in rats: V. Unilateral lesions have different behavioral and anatomical effects than bilateral lesions. *Psychobiology*, 17, 363-369.
- Kromer, L. F. (1987). Nerve growth factor treatment after brain injury prevents neuronal death. *Science*, 235, 214-216.
- 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.
- Lopez-Garcia, C. (1993). Postnatal Neurogenesis and regeneration in the lizard cerebral cortex. In A. C. Cuello (Ed.), *Restorative Neurology* (Vol. 6, Neuronal Cell Death and Repair, pp. 237-246). Amsterdam: Elsevier.

- 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*, 83, 2448-2452.
- Nottebohm, F. (1985). Neuronal replacement in adulthood. In F. Nottebohm (Ed.), *Hope for a new neurology* (Vol. 457, pp. 143-161). New York: New York Academy of Sciences.
- Nottebohm, F., & Alvarez-Buylla, A. (1993). Neurogenesis and neuronal replacement in adult birds. In A. C. Cuello (Ed.), *Neuronal Cell Death and Repair*.
- Oliverio, A., & Shepherd, G. M. (1991). Neurons: The cells of thought. In P. Corsi (Ed.), *The Enchanted Loom*. New York: Oxford University Press.
- Rakic, P. (1985). Limits of neurogenesis in primates. *Science*, 227(4690), 1054-1056.
- RamonyCajal, S. (1933). *Histology, 10th Ed.* Baltimore: Wood.
- Robinson, T. E., & Kolb, B. (1999). Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine and cocaine. *European Journal of Neuroscience*, 11(5), 1598-1604.
- Rowntree, S. (1995). *Basic Fibroblast Growth Factor in the Injured Brain*. Unpublished M.Sc., University of Lethbridge, Lethbridge.
- Rowntree, S., & Kolb, B. (1997). Blockade of bFGF retards recovery from motor cortex injury in rats. *European Journal of Neuroscience*, 9(11), 2432-2441.
- Schanberg, S. M., & Field, T. M. (1987). Sensory deprivation stress and supplemental stimulation in the rat pup and preterm human neonate. *Child Development*, 58,

1431-1447.

- Shankle, W. R., Landing, B. H., Rafh, M. S., Schiano, A., Chen, J. M., & Hara, J. (1998). Evidence for a postnatal doubling of neuron number in the developing human cerebral cortex between 15 months and 6 years. *Journal of Theoretical Biology*, *191*, 115-140.
- Sholl, D. A. (1956). *The organization of the cerebral cortex*. London: Methuen.
- Smith, A., & Sugar, O. (1975). Development of above normal language and intelligence 21 years after left hemispherectomy. *Neurology*, *25*, 813-818.
- Solkoff, N., & Matuszak, D. (1975). Tactile stimulation and behavioral development among low-birthweight infants. *Child Psychiatry and Human Development*, *6*, 33-37.
- Stewart, J., & Kolb, B. (1988). The effects of neonatal gonadectomy and prenatal stress on cortical thickness and asymmetry in rats. *Behavioral and Neural Biology*, *49*, 344-360.
- Thomas, K. A. (1987). Fibroblast growth factors. *FASEB Journal*, 434-440.
- Trice, J. E., & Stanfield, B. B. (1986). Evidence for the generation in the adult rat dentate gyrus of neurons that extend axonal projections. *Annals of Neurology*, *20*, 392.
- Weiss, S., Reynolds, B. A., Vescovi, A. L., Morshead, C., Craig, C. G., & VanderKooy, D. (1996). Is there a neural stem cell in the mammalian forebrain? *Trends in Neurosciences*, *19*(9), 387-393.
- Werker, J. F., & Tees, R. C. (1999). Influences on infant speech processing: Toward a new synthesis. In J. T. Spence, J. M. Darley, & D. J. Foss (Eds.), *Annual Review*

of Psychology (pp. 509-535). Palo Alto: Annual Reviews.

Whishaw, I. Q., Dringenberg, H. C., & Pellis, S. M. (1992a). Spontaneous forelimb grasping in free feeding rats: Motor cortex aids limb and digit position. *Behavioral Brain Research*, *48*, 113-125.

Whishaw, I. Q., Kolb, B., & Sutherland, R. J. (1983). The analysis of behavior in the laboratory rat. In T. E. Robinson (Ed.), *Behavioral Approaches to Brain Research*. New York: Oxford University Press.

Whishaw, I. Q., Pellis, S. M., Gorny, B., Kolb, B., & Tezlaff, W. (1993). Proximal and distal impairments in rat forelimb use in reaching following unilateral pyramidal tract lesions. *Behavioral Brain Research*, *56*, 59-76.

Whishaw, I. Q., Pellis, S. M., & Gorny, B. P. (1992b). Medial frontal cortex lesions impair the aiming component of rat reaching. *Behavioral Brain Research*, *50*, 93-104.