EFFECTS OF BEHAVIORAL THERAPIES AND PHARMACOLOGICAL INTERVENTION IN BRAIN DAMAGE

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DEDICATION

To my parents, Donna and Dick Witt, who acted as the bows and shot me off as the arrow...to my children, Lisa and Sarah, who are the arrows to my bow...and to that amazingly perfect structure, the brain.....

ABSTRACT

Maximizing recovery of function after brain injury is the goal for many neuroscientists and rehabilitation medicine professionals alike. To further elucidate the neural mechanisms underlying compensatory changes in brain injury and to determine the possibility of enhancing these changes, three experiments are described. Experiment 1 looks at the effects of structured (skilled reaching) versus functional (enriched environment) training with and without FGF-2, a pharmacological intervention, as treatment paradigms for rehabilitation-induced recovery of function in cortical lesion adult rats. Experiment 2 examines the treatment effects of tactile stimulation to enhance motor abilities in postnatal day 4 rat pups sustaining cortical damage. Finally, experiment 3 explores changes in the cortical motor representation after cortical damage. Results indicate a marked improvement on behavioral testing combining FGF-2 and functional training. Tactile stimulation significantly enhances recovery of motor functions. Post-lesion cortical mapping reveals changes in the motor representation utilizing the adjacent posterior parietal cortex.

ACKNOWLEDGEMENTS

"It all began the day I found that from my window I could only see a piece of sky. I stepped outside and looked around and never dreamed it was so wide or even half as high"...

from "A Piece of Sky" sung by Barbra Streisand

With much fondness and respect, I would like to acknowledge my supervisor, Dr. Bryan Kolb, who has provided me with the opportunity to explore the intricacies of the brain in a microscopic manner, and who has the rare gift of making the complex appear simple; and Dr. Gwendolyn Jansma who, over the years, continues to remind me to see the bigger picture.

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LIST OF ABBREVIATIONS

AchE	**Acetylcholinesterase**
ANOVA	**Analysis of variance**
bFGF	**Basic fibroblast growth factor**
CIT	**Constraint induced therapy**
CT	**Control treatment**
CNS	**Central nervous system**
E	**Embryonic day**
EE	**Environmental enrichment**
FGF-2	**Basic fibroblast growth factor**
FL	**Forelimb area**
Fr 1, 2, 3	**Frontal cortex, areas 1 (primary motor cortex), 2, 3**
FT	**Functional treatment or functional training**
Gu	**Gustatory cortex**
HL	**Hindlimb area**
Hz	**Hertz**
icv	**Intracerebral ventricular**
ip	**intraperitoneal**
kΩ	**Killiohms**
LSD	**Least significant difference**
LTD	**Long-term depression**
LTP	**Long-term potentiation**
ms	**Millisecond**

µA	**Microamps**
µg	**Microgram**
µm	**Micrometer**
NGF	**Nerve growth factor**
NMDA	**N-methyl-D-aspartate**
NT	**Non-treatment or non-training**
NTF	**Neurotrophic factor**
Oc 1B	**Occipital cortex, area 1, binocular part (primary visual cortex)**
Oc 2L	**Occipital cortex, area 2, lateral part**
Oc 2 ML	**Occiptal cortex, area 2, mediolateral part**
P	**Postnatal day**
Par 1	**Parietal cortex, area 1 (primary somatosensory cortex)**
Par 2	**Parietal cortex, area 2 (supplementary somatosensory cortex)**
RSA	**Agranular retrosplenial cortex**
rt-PA	**Recombinant tissue plasminogen activator**
SEM	**Standard error of the mean**
ST	**Structured treatment or structured training**
Te 1	**Temporal cortex, area 1 (primary auditory cortex)**
T	**Treatment or training**
TMS	**Transcranial magnetic stimulation**

EFFECTS OF BEHAVIORAL THERAPIES AND PHARMACOLOGICAL INTERVENTION IN BRAIN DAMAGE

1. GENERAL INTRODUCTION

A.B[1]., a professional, age 51, was admitted to the hospital after he had spent what he thought would be a typical day of doing yoga, then swimming prior to work. He noticed that he was swimming in circles, unable to break the pattern and realized he had had a stroke. Upon arriving within three hours to the hospital, he was diagnosed with a 1.2 cm right thromboembolic stroke in the white matter lateral to the right lateral ventricle. A carotid ultrasound indicated carotid artery flow to the brain was adequate. Medical history included borderline hypertension and hypercholesterolemia. He had reduced upper extremity function, reduced memory and mild slurred speech. Because he had arrived at the hospital within three hours, he was seen by the neurologist and had the opportunity to be involved in an international double blind drug study whereby he received either a placebo or gavestinel, an antagonist of the glycine site of the NMDA receptor. This medication, designed to act as a neuroprotectant, was being tested to determine efficacy in reducing secondary brain damage and improve functional outcome.

C.D., a manual laborer, age 53, on the other hand, was working outside when he sustained an accidental hit with a steel pipe in the area above the right eye. Visual difficulties and dizziness ensued in the following hour and he was taken to the hospital emergency department. He was sent home after testing

[1]The case histories are actual patients who were assessed and treated by the Post Acute Rehabilitation Team at the Lethbridge Regional Hospital.

and observation revealed no other symptoms. That evening he developed weakness on his left side, fell out of bed, and was readmitted to the hospital with left side paralysis. He was diagnosed with a thromboembolic stroke in the area of the right temporal horn, medial temporal lobe, and basal parietal/temporal region. He demonstrated left visual neglect, swallowing difficulties, slurred speech, left side motor and sensory impairments, and increased impulsiveness. A carotid ultrasound revealed that the right internal carotid artery was almost totally occluded, affecting middle cerebral artery blood flow and causing the resulting large infarct. C.D. was unable to reach the hospital in time to be a part of the drug study. A.B. was able to resume his employment part-time; C.D. was left with disabling left-side arm and leg weakness, and cognitive deficits. It is unknown, however, whether the drug treatment was a factor in A.B.'s post-stroke improvement.

What has allowed one individual to recover more completely from his brain insult than another? The answer lies in a combination of factors, and it is improbable to pinpoint any one factor as causing the damage. Taken together, these factors produce a variety of different chemical cascades in the brain, affecting individuals to varying degrees. This thesis contains three experiments that examine effects of behavioral treatments and cortical changes on recovery of function after brain damage.

1.1. WHAT INFLUENCES FUNCTIONAL RECOVERY IN BRAIN INJURY?

Ultimately, factors can be placed into two categories: those influencing the internal environment or the external environment (Table 1.1). Similar scenarios to those identified have happened in hospitals across the country. New awareness and subsequent advances in technology, both in the animal lab and clinical settings, have resulted in the ability to help the brain repair itself. In addition to standard behavioral therapies, novel treatments are occurring that involve drugs intended to reverse the effects of stroke or traumatic brain injury. To date, only recombinant tissue plasminogen activator (rt-PA), a thrombolytic agent designed to dissolve blood clots, has provided evidence deemed useful in successfully reperfusing ischemic brain tissue in humans. Other clinical trials are underway to determine efficacy of neuroprotectants that have been shown to be effective in lab animals (Lindsberg, Roine, Tatlisumak, Sairanen, & Kaste, 2000). The advantage of using lab animals is that we are able to limit the variables that induce brain damage and thereby determine how to get rid of or minimize the ongoing symptomatology of the damage, as well as determine the possible mediating factors underlying the damage in the first place.

Table 1.1. Internal and External Factors Influencing Recovery

Internal Environmental Influences	External Environmental Influences
Genetic Predisposition Co-morbid medical conditions Severity of insult Number of insults Area of insult Kind of brain trauma Medications/drugs Diet	Age at time of insult Pre-morbid educational background Motivation Stress Awareness of deficits Emotional factors Pre and post environmental experiences Extent and quality of rehabilitation

Note: Adapted from Kapur, 1997, p. 410.

Immediately, the consequences of brain damage hugely affect individuals, families, and society as a whole, and maximum recovery of function becomes the goal. It is with this goal in mind that we begin to look at plasticity in both human and animal models overall, then, specifically in relationship to recovery of function from brain injury.

1. 2. BRAIN PLASTICITY

Plasticity is the ability of the brain to reorganize its circuitry in response to experience or sensory stimulation. The brain and environment communicate interactively, profoundly influencing each other in a bi-directional manner. This bi-directional communication occurs at the level of the neuron, and dendrites, dendritic spines, and axons are continuously being modified (Figure 1.1). Hebb (1949) has been credited with hypothesizing that neuronal connections can be remodeled and become more efficient by our experience, particularly at the level

of the synapse, although the issue of whether the environment can affect the brain fascinated scientists and philosophers far earlier. For example, Rosenweig (1979) described the work of Michele Vincenzo Malacarne, a Piedmontese anatomist from the 1700s, who correlated individual differences in humans with differences in brain structure. In order to test this hypothesis, one of his experiments utilized pairs of parrots, chaffinches and blackbirds. One bird received extensive training for a number of years, while the other was untrained. When sacrificed, the trained birds had more folds in the cerebellum than the untrained counterparts.

Subsequent research (Bennett, Diamond, Krech, & Rosenweig, 1964) has shown that, indeed, there are chemical and anatomical plastic changes occurring in the brain due to enriched experiences in rats, particularly greater weight and thickness of cortical tissue and an increase in total acetylcholinesterase. The increase in this enzyme implies an increase in acetylcholine, a neurotransmitter involved in learning and memory. Morphological structures such as dendritic branching, and an increase in synapses per neuron, were altered by housing rats in an enriched environment versus an impoverished one (Diamond et al., 1966). Similar studies in enriched environments show changes in gene expression, and local neurotrophin action, chemicals that support neuronal survival and growth (Klintsova & Greenough, 1999). Standard motor learning tasks, not mere motor activity, produced similar changes (Kleim, Lussnig, Schwartz, Comery & Greenough, 1996). Neeper, Gomez-Pinella, Choi, and Cotman (1995) showed that plastic changes occur as a result of physical activity, which increases

neurotrophic gene expression in specific brain regions. Researchers demonstrated that cortical representation areas or cortical maps could be changed by experience and learning, and lesion induced and use-dependent plasticity could restore lost function in brain injury (Merzenich et al., 1983; Nudo, Wise, SiFuentes, & Milliken, 1996). Finally, evidence is accumulating to show that this plastic reorganization occurs throughout the lifetime of the individual, but occurs in different areas of the brain and at different rates depending on the age of the animal (Kolb, 1995).

Plasticity, then, occurs under four main conditions: 1) Developmental plasticity-when the immature brain begins to process sensory information, which can vary depending on the embryonic development of the species; 2) Activity-dependent plasticity-when sensory information is altered in the brain due to such changes as visual acuity, auditory acuity, drug addiction or exercise of specific body parts; 3) Plasticity of learning and memory-when behavior is changed as a result of new sensory information; 4) Injury-induced plasticity-changes following brain insult (John F. Kennedy Center for Research on Human Development, Vanderbilt University, 2000).

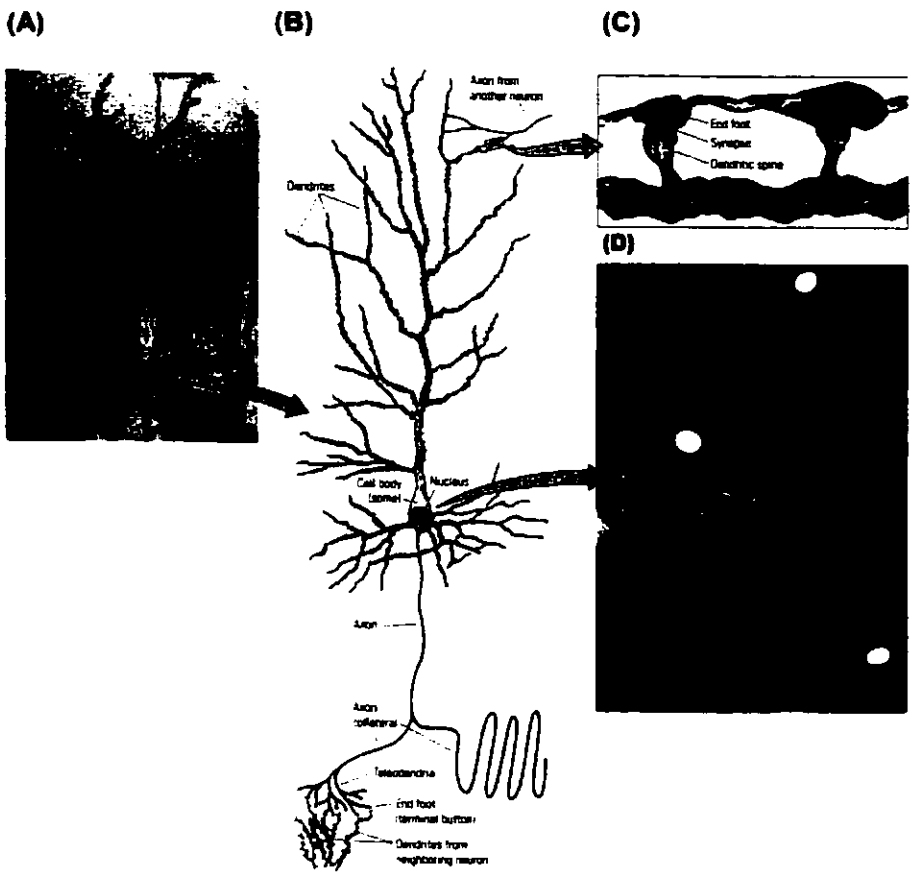


Figure 1.1. The major parts of a neuron. **(A)** A typical neuron that is stained by using the Golgi technique. **(B)** A drawing of a neuron showing its major physical features. **(C)** An electron micrographic image of the contacts between an axon of one neuron and a dendrite of another. **(D)** A high-power light-microscopic view of the cell body (From Kolb & Whishaw, 2001, p. 81).

1. 3. POSSIBLE NEURAL MECHANISMS UNDERLYING BRAIN PLASTICITY

In the neuroscience field, it is generally thought that the same neural mechanisms underlie all four types of plasticity and that the activity occurs primarily at the synapse. Johansson (2000) provides a general review of

possible neural mechanisms underlying brain plasticity. First, long-term potentiation (LTP) and long-term depression (LTD) are long-lasting synaptic alterations that follow brief electrical stimulation induced in various areas of the brain. These are activity-dependent and it is thought that information is strengthened pre- and postsynaptically and stored through these processes in the central nervous system (Bliss, 1993; Bear & Malenka, 1994). Second, synaptic plasticity in cortical horizontal connections has been proposed to underlie cortical map reorganization. Glutamate is the main excitatory neurotransmitter and is thought to play a pivotal role in plasticity, particularly in relationship to the N-methyl-D-aspartate (NMDA) receptor complex (Hess, Aizenman, & Donaghue, 1996). Third, local neurotrophin actions and synaptic protein synthesis are thought to promote synaptic remodeling changes (Klintsova & Greenough, 1999). Fourth, there can be rapid changes occurring at dendritic spines, which may be due to the presence of actin at the postsynaptic site (Fischer, Kaech, Knutti, & Matus, 1998). Fifth, in vitro studies show that glial cells promote the formation of synapses, take up released transmitter and provide energy substrates and neurotransmitter precursors to synapses, thus helping to maintain their proper function (Pfrieger & Barres, 1996). Kolb (1999) discusses a theoretical model that incorporates both the role of the neuron as well as the importance of the neuronal environment in plasticity. All of the above stated neuronal substrates fit into Kolb's ecological theory.

1. 4. PLASTICITY AND NORMAL AGING

Kolb reported that the plasticity that is available to the brain varies depending on the age of the animal. He and colleagues have spent years developing experiments to determine those periods of time when rats are most and least susceptible to neural plastic changes (for a review see Kolb, Forgie, Gibb, Gorny, & Rowntree, 1998a). Based on these experiments, general guidelines were established as to when certain specific cellular events took place and corresponding time-dependent differences in cortical plasticity occurred (Figure 1.2)

Neuronal birth in the rat occurs at ~ embryonic day 12 (E12) and continues until ~ E21 with birth occurring on E22 (Uylings, Van Eden, Parnavelas, & Kalsbeek, 1990). As the figure indicates, that time period when there is astrocytic proliferation, dendritic growth, synapse formation, and spine growth, from ~ post-natal day 7 (P7) until ~ P14, also corresponds to that time period when there is the most plasticity. From P15-30 is a period where there is neuronal death and synaptic pruning so that plasticity may be greater than in the adult. Another period of plasticity occurs at ~ P60, as rats reach puberty and gonadal hormones influence cell structure and connectivity.

Plasticity continues to decline gradually until senescence, when the drop is more dramatic. Nonetheless, even the senescent brain is plastic. For example, senescent rats housed in an enriched environment have thicker regions of cerebral cortex and cerebellum compared to animals housed in standard cage housing (Black, Greenough, Anderson, & Isaacs, 1987). Studies in the Kolb lab

have shown that older rats housed in enriched environments had greater spine density than middle aged rats. The speed of the plastic changes, however, is reduced relative to younger animals.

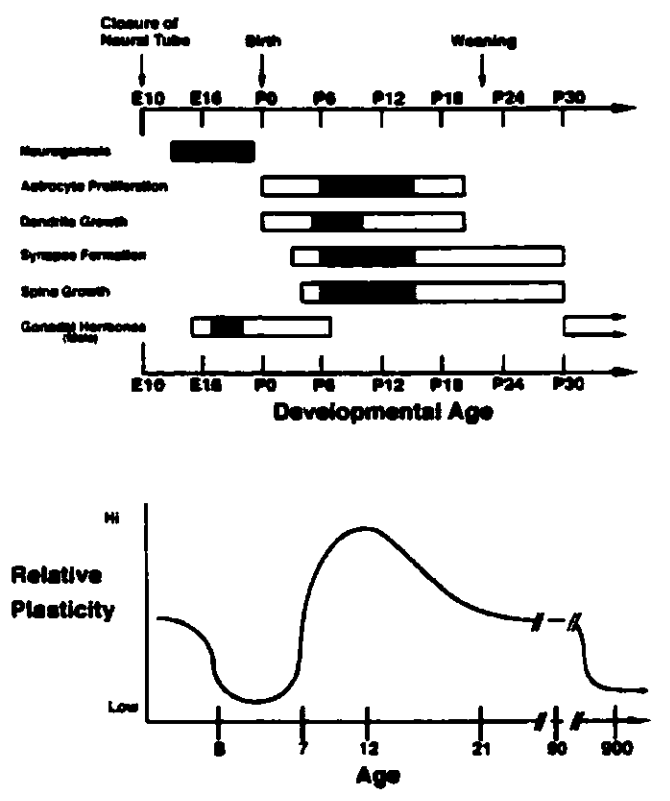


Figure 1.2. Top. Main cellular events related to cortical plasticity. Bars mark the approximate beginning and ending of different processes. The intensity of the shading reflects the intensity of the phenomenon. **Bottom.** Summary of the time-dependent differences in cortical plasticity (From Kolb, 1999).

In addition to neurogenesis in the embryonic phase, there is continuous neuronal generation in the area of the olfactory bulb and dentate gyrus of the

hippocampus throughout life. It is known that the new neurons in the olfactory bulb originate from stem cells in the subventricular zone, and it is possible that these stem cells also may produce neurons for other places in the brain. Because of this continuous stem cell production, it is theoretically possible for neurogenesis to occur throughout life if the right conditions are present. This phenomenon has implications in producing plastic changes after recovery of function from brain injury.

1. 5. PLASTICITY AND RECOVERY OF FUNCTION FROM BRAIN INSULT

1. 5. 1. What does recovery of function mean?

"Recovery of function" has different definitions depending on whether one works in the clinic with people, the lab with animals, or is the recipient of the brain insult (Kolb, 1995). As a rehabilitation specialist (speech-language pathologist), my initial idealistic thought in working with people was to help them recover completely from their brain damage. I soon learned that what I was probably doing was helping individuals compensate and adapt to their loss, such as providing a change in strategy or substituting a new behavior for the lost one. Partial restitution of the original behavior is another possible outcome after brain injury. So it may be that recovery is occurring due to a reduction in swelling, for example, or that there can be an actual return of function due to plastic changes in the brain. The former example would occur much more quickly (hours) than the latter one (months). Another possibility is complete restitution of the original behavior. Despite the fact that it is theoretically possible that functions can return

completely after brain damage, careful behavioral analysis suggests that the return is most likely due to compensatory behaviors. One final interpretation of recovery is that a certain treatment such as a drug might help the return of function, and that without that drug treatment the behavior would not occur, or the recovery process would not occur as quickly. The treatment then, might make it easier to perform the behavior and to prevent further loss. Recovery, in this case, may mean less behavioral loss.

As a researcher then, my goal is on "recovery of function" (complete restitution). As a clinician, my goal is to maximize "recovery of function", but often settling for "compensation." From a client's point of view, however, complete recovery of function is the goal, and *any* recovery may be interpreted by the client as evidence of complete recovery.

1. 5. 2. Cortical Plasticity and Brain Injury

As stated earlier, recovery from injury is dependent upon a variety of factors. These factors can include, timing of the central nervous system (CNS) response to injury, age at time of injury as well as location, type, and size of injury. Treatment administration factors include type, frequency, intensity, duration, and when to initiate. For the purposes of this thesis, I will focus mostly on studies involving cortical lesions and to touch on each of the above factors.

1. 5. 2. 1. Timing of the CNS response to injury

After the brain is injured there are three possible ways that repair can occur: reorganization of existing circuits, production of chemical messengers to enhance reorganization of existing circuits, and the creation of new neurons and thus new circuits (Kolb et al.,1998a). Cotman and his colleagues (1985) detailed a strict time-dependent course of cellular and molecular events that occurs in the brain following CNS damage in the dentate molecular layer after unilateral entorhinal ablation in adult rats. All these events occur almost simultaneously once the process is initiated (Figure 1.3).

In this model, the following sequence occurs after the primary CNS injury: 1) During the first few days, axonal fiber and synaptic degeneration, and clearing of debris takes place. 2) Vascularization, repair of the broken blood-CNS barrier, and a new glial boundary occurs. The blood-CNS barrier is necessary so that metabolites such as glutamate and aspartate do not flood all the excitatory synapses. 3) Secondary injury is in progress at this point, reaching a maximum around 4 days after the lesion injury. 4) Reactive growth can be observed starting at 4 days postlesion. This injury induced synaptic formation is called reactive synaptogenesis and this process provides presynaptic input to damaged deafferented neurons, which then prevents dendritc atrophy; and 5) Maximum neurotrophic factor production is reached 8-10 days postlesion. The priority, then, of the organism is directed towards overall survival before the restoration of functional circuitry. By the time that events occur for restorative purposes, regenerated sprouts cannot grow through the newly developed glial boundary.

This information is helpful in determining what molecular mechanisms may be modified to ameliorate brain injury.

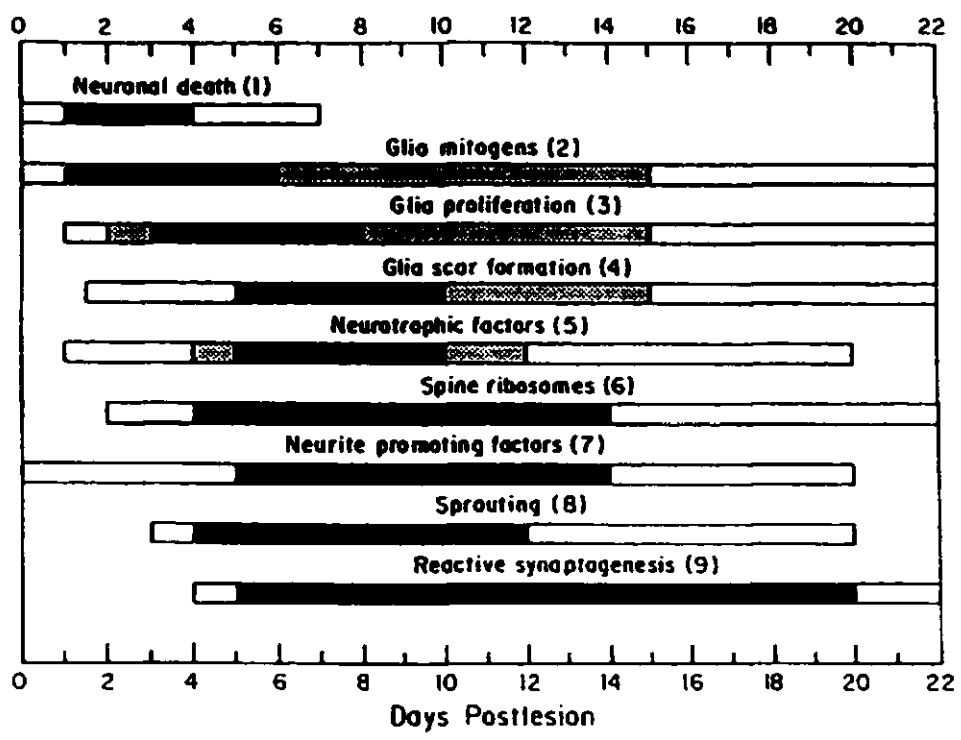


Figure 1.3. Time course of events following CNS injury. The beginning of the bars indicates when the event is initiated. Many of these events continue beyond the time scale covered in the graph. The intensity of the shading parallels the intensity of the phenomenon indicated above the bar. Note that regeneration promoting responses (4-9) occur simultaneously, reaching maxima around 7-10 days postlesion (After Nieto-Sampedro & Cotman, 1985, p. 440).

1. 5. 2. 2. Age, location, type, and size of injury

1. 5. 2. 2. 1. Age

Kolb and colleagues have defined four distinct types of cortical plasticity in injury occurring at various ages based on several decades of studies (e.g.: Kolb et al., 1998a). As research continues these results are generally consistent depending on the location of the damage; 1) If the injury occurs during neurogenesis, which is *in utero* in the rat, the functional recovery is good, but the cortical morphology is unusual in its structure; 2) If the injury occurs from P1-P6 the functional recovery is poor and this is correlated with neuronal atrophy; 3) If the injury occurs from P7-P12, there is good functional recovery, dendritic and spine growth, and if the damage is in the medial frontal area of the brain, then there is actual neurogenesis and brain regrowth; 4) If the injury is in adulthood, there is initial dendritic atrophy, then growth with partial return of function. In conclusion, if there is no synaptogenesis, there is no functional recovery, but if there is synaptogensis there is at least partial recovery.

1. 5. 2. 2. 2. Location

Behavioral manifestations of cortical injury vary depending on the location of the brain insult. In adult humans, for example, damage to specific areas in the left frontal or temporal lobes will cause an individual to have difficulties in speech production or comprehension of speech, respectively. A totally different type of speech disorder called dysarthria or slurred speech would occur if damage was in a specific area of the cerebellar cortex. Behavioral and anatomical effects have been examined in humans, laboratory monkeys, and rats with damage to

various areas of the brain, both in just one hemisphere and then in both (Lee & Donkelaar, 1995; Kolb & Whishaw, 1996; Steinberg & Augustine, 1997).

With regard to lesions restricted to the motor cortex, it is generally found that lesion effects that are unilateral affect the contralateral limb on skilled reaching and other tests of motor function. If lesions are larger, then there can be a bilateral effect of the lesion and reaching performance may be affected in both limbs (Kolb, Cioe, & Whishaw, 2000a). The degree of damage to corticospinal connectivity appears to be a factor in the degree of recovery, with better recovery occurring if there remain some corticospinal connections.

In bilateral motor cortex lesions there are residual behavioral effects that are constrained to the motor domain in young (P10) and adult lesion animals, but there are more generalized motor and cognitive disabilities in younger animals (P1-4) tested when adults (Kolb & Holmes, 1983; Kolb et al., 2000a). Motor behaviors that are affected include skilled forelimb reaching, food manipulation, tongue protrusion, claw cutting, and beam traversing (Kolb & Holmes, 1983; Kolb & Whishaw, 1983; Kolb et al., 2000a).

1. 5. 2. 2. 3. Type

The type of brain injury also will affect overall behavioral function. For example, a traumatic brain injury caused by a motor vehicle accident will produce very different symptoms from a stroke. In the former scenario, the damage may be localized or diffuse, depending on where the impact of injury is. In the latter scenario, the symptoms seen will be more localized to specific deficits, depending on which hemisphere was damaged.

In animal research there are several models to study effects of recovery from motor cortex damage. In the Kolb lab, the majority of lesions are done using gentle aspiration of cortical tissue. Another major model used in studying stroke is a middle cerebral artery occlusion induced by electrocoagulation of the middle cerebral artery. Interestingly, behavioral data from studies comparing various motor and cognitive tests in rats reveal that there is little difference between the above two types of lesions made (C. Gonzales and B. Kolb, unpublished observations). This finding suggests that rehabilitation-induced functional improvement can be examined using the aspiration method of lesioning, and that this method can also provide valuable insights into neural mechanisms underlying brain insult.

1. 5. 2. 2. 4. Size

Recovery is related to cortical lesion size, although again, other variables such as location and age at injury can affect the overall outcome. For example, if a child has a left hemispherectomy for relief from seizures at the age of 1 year or older, speech and language skills can develop because these skills shift to the right hemisphere (Vargha-Khadem, Watters, & O'Gorman, 1985). In fact, this behavioral compensation has been shown to occur in a child who did not develop speech and language until after left hemidecortication at the age of 8.5 years (Vargha-Khadem, Carr, et al., 1997). This case study supports the idea that a "bad brain is worse than no brain," and that the intact hemisphere can compensate for some of the damage done to the lesion hemisphere if the damaged area is removed. Vargha-Khadem et al. (1985) also reported that

children with perinatally acquired bifrontal injuries have persistent severe speech and language deficits, presumably due to the inability for the speech and language centers to shift to the right hemisphere.

This lack of hemispheric transfer was supported in an animal study by Kolb (1992). When infant rats were given hemidecortication, followed by a small stab wound in the opposite hemisphere, there was neither behavioral sparing nor anatomical changes in the remaining hemisphere that would normally be seen with this type of lesion. However, complete decortication in adult rats precludes much recovery of function (Whishaw, 1990), and neonatal decortication allows little sparing of function (Whishaw & Kolb, 1989).

Whishaw (2000) performed a series of experiments examining postlesion reaching success and reaching movements after five different unilateral motor cortex lesion sizes. Although impairment was generally proportional to lesion size, individual responses by animals were variable. It was hypothesized that the lesions resulted in a loss of the cortical engram or cortical substrate that supports species-typical skilled movements and that recovery was due to compensatory movements mediated by remaining brain areas.

The issue of unilateral versus bilateral lesions was previously mentioned in relationship to location. Hicks and D'Amato (1970), in a series of seminal studies, made unilateral motor cortex lesions and showed that recovery was dependent upon the presence of abnormal ipsilateral corticospinal projections from the normal hemisphere. Studies by Kolb et al. (2000a, 2000b) examined recovery after bilateral and unilateral motor cortex lesions, respectively. Overall,

these and other studies (e.g.: Jones & Schallert, 1992, 1994; Jones, Kleim, & Greenough, 1996) indicate that there is better behavioral recovery if there are some remaining corticospinal connections in the damaged cortex or an intact contralateral hemisphere.

1. 5. 2. 3. Treatment Administration Factors

Recovery of function also depends upon various treatment administration factors including type, frequency, intensity, duration, and when to initiate treatment. Given the enormous evidence that behavioral experience affects brain structure and vice verse, it is reasonable to assume that various behavioral therapies would affect overall recovery of function. The general rule in clinical rehabilitation is to begin treatment as soon as possible after the insult, with maximum frequency, intensity and duration of treatment. However, actual evidence that there are changes in neural structures and/or improved functional outcome as a result of rehabilitation is only beginning to be shown in good animal and clinical research based studies (Kozlowski, James, & Schallert, 1996; Cifu & Stewart, 1999; Liepert, Bauder, Miltner, Taub, & Weiller, 2000; Jorgensen et al., 2000; Biernaskie & Corbett, 2001). For purposes of this thesis, only treatment type will be discussed.

1. 5. 2. 3. 1. Treatment type

In the clinical setting, most rehabilitation therapy, after thorough diagnostic testing, consists of treatments that contain both a structured and functional format. These treatments, including speech, physical, and occupational therapies, may occur approximately an hour per day for each discipline. For

example, a structured approach might include a stimulus/response activity in right upper limb motor training such as putting pegs from the left visual field to the right, whereas a functional activity would include practical applications during motor training such as writing a letter. In animal studies, the structured/functional dichotomy is differentiated by specific training tasks versus generalized experience through complex cage housing.

Three primary types of animal models that are used to research rehabilitation-induced functional improvement from cortical damage include: enriched environment, skilled reaching training, and tactile stimulation. Environmental enrichment (EE) was first reported by (Hebb, 1949) who discovered that rats living in a free environment performed better on tasks than those animals living in a restricted environment. Researchers in Berkeley (Bennett et al., 1964; Bennett, 1966) subsequently found that animals housed in enriched environments had chemical, neuroanatomical and behavioral alterations compared to those housed in standard lab cages. These changes included increases in brain weight, neuron size, dendritic spines, glial proliferation and cortical cholinesterase activity. However, changes in neural structures were dependent upon how the animals interacted with the environment, (for a review, see Will & Kelche, 1992). Overall, subtle differences were seen in the manner in which animals accessed and utilized information from the environment compared to standard cage housing. The EE cages generally consisted of a large wire mesh enclosure containing items such as ladders, boxes, trampoline, rope, and other objects with which to interact both horizontally and vertically. Studies using

EE as a treatment to examine functional outcome in brain injury have shown functional improvement, even if animals with middle cerebral artery occlusion lesions were transferred into the EE situation after 15 days (Johansson, 2000). Kolb (1999) has shown that animals with various lesions at different ages and being in the EE housing demonstrate marked morphological, anatomical, and behavioral changes compared to animals in standard cage housing.

The second type of animal model used to look at rehabilitation-induced functional improvement is the skilled reaching model. Skilled reaching generally consists of the animal reaching through a cage with the affected limb in attempts to successfully acquire food pellets. Nudo, et al. (1996) reported that adult squirrel monkeys given motor cortex lesions were able to improve skilled hand function by receiving intensive retraining in skilled hand use to retrieve food from small wells. When performing cortical mapping using intracortical microstimulation techniques, researchers discovered functional reorganization had occurred in the undamaged motor cortex adjacent to the infarct. Subsequent skilled reaching studies with rats showed functional changes corresponding to anatomical changes in the damaged cortex using the same cortical mapping techniques (Kleim, Barbay, & Nudo, 1998). These results indicated that rehabilitative training using skilled reaching could change anatomical structures and improve functional outcome.

Finally, the third type of animal model used to examine recovery of function is tactile stimulation. This form of stimulation has its roots in the neonatal handling techniques introduced in the 1950s (Levine, 1957). This early

procedure consisted of rat pups being removed from the dam daily for 3-15 minutes until weaning (~ P21), but not necessarily being handled or touched for that amount of time. Subsequent studies involving actual handling showed that this form of treatment stimulated growth and endocrine changes in premature infants (Field et al., 1986) and newborn rats (Schanberg & Field, 1987). The tactile stimulation technique in the Kolb lab involves stimulating rat pups three times per day for 15 minutes each time with a camelhair brush until weaning. Studies by Gibb (2001) have shown that there are morphological changes and functional recovery using tactile stimulation techniques in animals given frontal/parietal lesions at P4 and frontal lesions at P3.

1. 6. NEUROTROPHIC FACTORS

Attempts to find a neuroprotectant that can minimize the anatomic and physiological disruptions from brain insult has been a goal for laboratory and clinical researchers for years. As previously stated, rt-PA is currently the only drug that is used clinically to help minimize the effects of an acute CNS injury, the result being a dissolving of the blood clot, or thrombolysis in an ischemic event (DeGraba & Pettigrew, 2000). There is, however, a region of brain surrounding the central damaged area whereby the metabolic capacity is reduced but not yet destroyed. This area is called the penumbra, and the goal of neuroprotectants or other chemicals to stimulate the brain to repair itself would be to aid in minimizing the secondary neuronal cell death. One chemical that has

been considered for use in both neuroprotection and stimulation of brain repair has been basic fibroblast growth factor (bFGF or FGF-2).

FGF-2 is a neurotrophic factor (NTF), one of a group of compounds produced by the brain that is a promoter of dendritic and synaptic growth and differentiation and in some cases, overall neuronal survival. The first NTF, nerve growth factor (NGF), was discovered approximately 50 years ago, and found to be essential for the development of the peripheral nervous system. NGF is now thought to influence recovery from motor cortex damage (Kolb, Cote, Ribeiro-da-Silva, & Cuello, 1997). Trophic factors are produced by both neurons and glia and can be mediated through cell membrane receptors, or by entering the neuron and acting internally on its operation (Kolb & Whishaw, 2001).

NTFs have been found to increase in the area of the penumbra and the wound cavity following brain injury (Nieto-Sampedro, Manthorpe, Barbin, Varon, & Cotman, 1983). This time-dependent increase was correlated with an increase in glial cell proliferation, and the authors proposed that the glial cells produced the NTF. In 1988, Needels and Cotman found that cultured hippocampal neurons bathed in a variety of different NTFs survived most effectively when the NTF was FGF-2.

Subsequent studies by Rowntree (1995) and Rowntree and Kolb (1997) have shown that, indeed, FGF-2-reactive astrocytes are present following motor cortex injury in the tissue surrounding the lesion. There is a specific time course, whereby FGF-2 appears two days after injury and reaches a maximum seven days after the injury before declining. This result suggests that FGF-2 may be a

necessary component for recovery in brain injury. Neutralizing antibodies to FGF-2 block the increase of FGF-2-reactive astrocytes around the injury, which then block functional recovery. Finally, an increase in endogenous FGF-2 is found in lesion animals housed in both standard cage and enriched housing, but there is an enhanced response in the animals housed in the enriched environment.

Finklestein and colleagues at Harvard have focussed on both animal and clinical studies using FGF-2 therapeutically, mainly looking at infarct volume and functional recovery postlesion. They use an animal stroke model by occluding the middle cerebral artery through electrocoagulation when giving lesions. Studies have included administering either pre- or postlesion injections of FGF-2 through a variety of methods, including intracisternally, where the chemical moves into the subarachnoid space (Kawamata, Alexis, Dietrich, & Finklestein, 1996; Kawamata et al., 1997), intraventricularly (Koketsu et al., 1994), or intravenously into the femoral artery (Bethel, Kirsch, Koehler, Finklestein, & Traystman, 1997; Sugimori, Speller, & Finklestein, 2001). Generally, they have found that administration of FGF-2 within the first three hours after the onset of ischemia is the effective time window for infarct size reduction. If FGF-2 is administered intracisternally starting at one day after ischemia, recovery of function is increased, but infarct volume remains the same. A summary of the studies done with animals using FGF-2 in cerebral ischemia can be found in a review by H. Ay, I. Ay, Koroshetz, and Finklestein (1999) with impressive decreases in infarct volume post lesion, ranging from 24-68%.

Clinical trials using FGF-2 began in 1997-98 (Internet Stroke Center, Stroke Trials Directory, 2001) in the United States and Europe. The phase II/III clinical study involved IV administration of the FGF-2 drug called trafermin within 6 hours of onset to individuals with an acute ischemic stroke. The study was halted in 1999 following an interim analysis, based on an unfavorable risk to benefit ratio in patients treated with the drug as opposed to the placebo.

1. 7. SUMMARY OF GENERAL INTRODUCTION

In summary, this introduction has pointed out several key issues in the recovery of function from brain damage.

1. Functional recovery is multifactorial and is influenced by both the internal and external environment.
2. Brain plasticity plays an important role in neuronal changes throughout life, and occurs under four main conditions: developmental plasticity, activity-dependent plasticity, plasticity of learning and memory, and injury-induced plasticity.
3. There are a number of possible neural mechanisms that underly brain plasticity, which are included in a general theoretical model by Kolb (1999). This model emphasizes the importance of the neuronal environment as well as the internal structures of the neuron itself as playing a key role in plasticity.
4. Plasticity varies in intensity depending upon the age of the organism.
5. "Recovery of function" has different meanings depending on whether one is a clinician, a researcher, or the recipient of the brain damage.

6. The degree of cortical plasticity after brain injury varies depending on timing of the CNS response to injury, age at the time of injury, location, type, and size of injury. Treatment administration factors affecting plasticity include type, frequency, intensity, duration, and when to initiate treatment.
7. There is a strict time dependent course of cellular and molecular events that occurs in the brain following CNS injury. The priority of the organism is first survival then restoration of cortical circuitry. Knowing when the events occur will assist in implementing treatments to ameliorate brain injury.
8. There are three primary types of animal models to research rehabilitation-induced functional improvement from cortical damage: environmental enrichment, skilled reaching, and tactile stimulation.
9. Neurotrophic factors (NTF) have been found to promote dendritic and synaptic growth. One type of NTF that has been studied extensively in animal and clinical studies is basic fibroblast growth factor (bFGF or FGF-2).

1. 8. THESIS CONTENT AND ORGANIZATION

This thesis contains three experiments that examine effects of behavioral treatments and cortical changes on recovery of function after brain damage. The fundamental questions are 1) whether structured versus functional experience will influence recovery; 2) whether the neurotrophic factor FGF-2 can influence recovery either alone or in combination with experience; and 3) whether experience in an infant with a similar injury will potentiate recovery. Experiment 1 involves differentiating between structured (skilled reaching) verses functional (enriched environment) training as rehabilitation treatment paradigms over four months in unilateral motor cortex lesion adult rats. An additional factor is the use of FGF-2 in the above groups to determine efficacy of this neurotrophic factor on recovery of function. A battery of tests was used to determine recovery over time, and to help establish an animal model to examine effectiveness of rehabilitation training. Experiment 2 explores tactile stimulation as a behavioral training paradigm and its effect on motor and cognitive functions in adult rats given bilateral motor cortex lesions at P4. Experiment 3 examines the results of cortical mapping in adult male rats having sustained bilateral motor cortex lesions at P4, trained in skilled reaching, and how the cortical map is changed compared to controls. These studies, then, examine the effects of rehabilitation-induced functional improvement over time given several factors: age, sex, lesion site, type of training, time of training and use of neurotrophins.

2. EXPERIMENT 1: BEHAVIORAL TREATMENTS, FGF-2, AND RECOVERY OF FUNCTION

2. 1. INTRODUCTION

As noted in the previous chapter, there are three primary phenomena with regard to recovery of function from brain damage. First of all, in the area of brain plasticity, there is converging evidence that structure begets function and vice versa in the interaction between the internal anatomical and morphological structures and external experience. Second, from an anatomical and morphological viewpoint, enriched experience in a complex environment increases overall brain weight, dendritic, spine, and synaptic complexity. Behaviorally, it improves motor abilities and cognitive status. Third, The neurotrophin FGF-2 has been implicated as a regulator in the response to neurological injury. In both animal and human studies, exogenous FGF-2 has been shown to enhance dendritic and synaptic growth and reduce infarct size concurrent with functional recovery.

In the initial phases of recovery, clinical rehabilitation treatments including speech, physical, and occupational therapies emphasize both a structured and/or functional approach for approximately an hour per day for each discipline. For example, a structured approach might include a stimulus/response activity in motor training, whereas a functional activity would include practical applications during motor training. In animal studies, the structured/functional dichotomy is differentiated by specific training tasks such as skilled reaching versus generalized experience through complex cage housing. Although evidence is

mounting, in neither human clinical studies nor animal studies is there much known about why or how such treatments and interventions might be effective.

The interplay between these treatment factors has not been examined. The purpose of this study, therefore, was twofold. Because it was uncertain whether there was a difference in the effect of structured (skilled reaching) versus functional (complex cage housing) training, this comparison was examined using unilateral motor cortex lesion adult rats. To determine the efficacy of FGF-2 in recovery of function and its interaction with behavioral training, half of the animals received the neurotrophin for 7 days post lesion, then were placed into either structured or functional training groups. Rehabilitation-induced recovery of function was assessed at specified intervals over four months using a battery of tests sensitive to motor cortex damage. The assessment protocol included a reaching task, spontaneous vertical exploration task, forepaw inhibition during swimming, tongue extension, single pellet reaching, and claw cutting. Cortical thickness and brain weights were measured to determine if there were anatomical effects of the prescribed treatments.

2. 2. METHODS AND PROCEDURES

2. 2. 1. Animals

The study was done with 54 adult male rats, 400-675 grams, from the Charles River Long-Evans strain. Animals were randomly assigned to one of three groups: control, unilateral motor cortex lesion, and unilateral motor cortex lesion with post-lesion infusion of FGF-2. Treatment groups consisted of non-

treatment (n=18), structured treatment (n=18) or functional treatment (n=18). Non-treatment (NT) and structured treatment (ST) groups were housed in hanging cages individually or in groups of four. Functional treatment (FT) groups were placed in complex cage housing measuring 1.8 m high X 1 m deep X 1.5 m wide in groups of five or six (Figure 2. 1). In the complex cage housing there were runways, platforms, rope, trampoline, and small hanging cages attached to the wire mesh front.

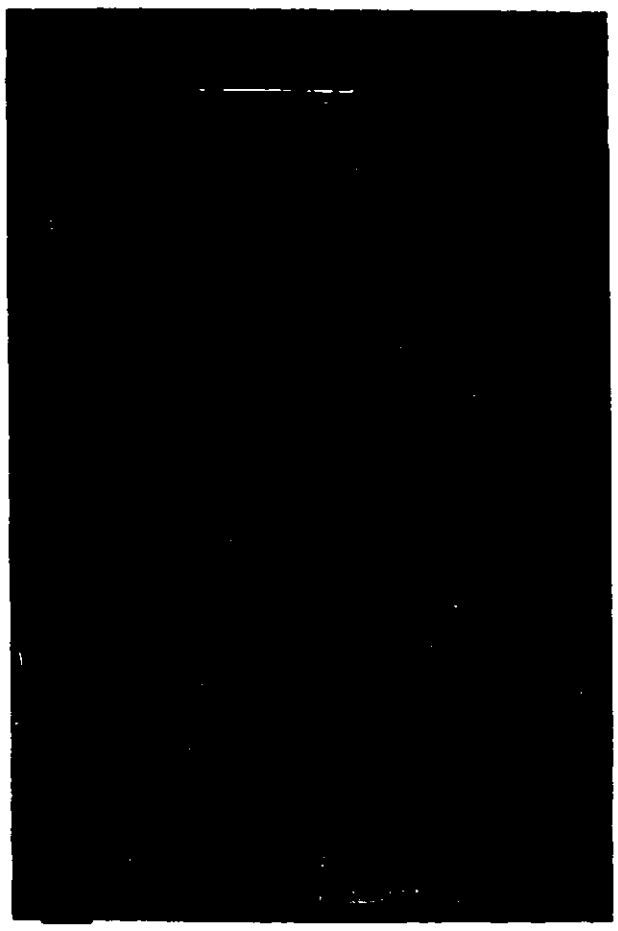


Figure 2. 1. Representation of complex cage housing in environmental enrichment studies (functional treatment).

All animals were maintained on a 12h light:12h dark cycle at a temperature of 22° C. They were given ad libitum food and water, except during training and testing, when they were food-deprived to 85% of their body weight. Animals receiving training in the complex cage housing were placed there several days prior to receiving surgery, in order to get accustomed to their environment.

2. 2. 2. Pre-training

Prior to being placed into treatment groups, all rats were pre-trained on the Whishaw reaching task (Whishaw, O'Connor, & Dunnett, 1986; Whishaw, Pellis, Gorny, & Pellis, 1991) to determine forepaw use and paw dominance. The preferred paw was then considered the dominant paw in subsequent training and testing protocols. Rats were trained and tested in nine Plexiglas cages (Figure 2.2). Each individual cage measured 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 one cm, edge to edge. A 20 cm wide and 5 cm deep tray containing chick feed was mounted on the front of each cage. The distance from the tray to the front of the box was 5 mm. To receive food, the rat had to reach through the bar and grasp the food. The base of the box was made of wire mesh, so that if the rat dropped the food, it fell and was lost through the mesh. Rats were trained 20 minutes per day until they all learned the task (approximately two weeks). After learning the task, they were then videotaped for 5 minutes on a Canon ES950 8 mm video camcorder and a halogen lamp

was used for lighting effects. Performance was examined afterwards on a Sony Trinitron Monitor and Sony EV-S900 NTSC Video Hi 8 video cassette recorder utilizing frame-by-frame analysis during the time of the reach. Reaching movements were analyzed by using the following categories: a 'reach' was recorded when the rat had touched the food, but had not been able to put it into the mouth; a 'hit' was counted when the rat was able to reach for and eat the food. Percentages were determined for each paw, and paw dominance was established.

Figure 2. 2. Whishaw reaching boxes.

2. 2. 3. Surgical Procedures

Lesion and lesion+FGF-2 rats were anesthetized with intraperitoneal (ip) injections of sodium pentobarbital (65 mg/kg) and atropine methyl nitrate (5 mg/kg). Surgeries were performed according to procedures described by Rowntree and Kolb (1997) and Kolb, Cioe, and Whishaw (2000b). Craniotomy was performed by removing the bone over the motor cortex from 1 mm lateral to the midline to the sagittal ridge (4 mm lateral) and from -4 mm bregma anterior to +2 mm bregma. After craniotomy and incision of the dura, focal unilateral suction lesions (6 mm long x 3 mm wide) were made. These aspiration lesions included Zilles' (1985) areas Fr1, the lateral part of Fr2, the posterior part of Fr3, and FL. Lesions were made in the motor cortex contralateral to the preferred paw. Animals were given FGF-2 according to similar procedures described by Kolb, Gorny, Cote, Ribeiro-da-Silva, and Cuello (1997). The animals received intracerebral ventricular (i.c.v.) infusions of FGF-2 for seven days via a minipump placed into the intact hemisphere immediately after lesions were performed. Stainless steel (23 gauge) cannulae were implanted into the intact lateral ventricle at the following coordinates relative to bregma: anterior/posterior, -0.8 mm; lateral, 1.5 mm; and, ventral, 3.5 mm from the skull. The cannulae were connected to sterile coiled polyethylene tubing filled with an air-oil spacer at the minipump end, and filled with 1 µg human recombinant FGF-2 (R & D Systems cat. 233-FB-025). The minipumps and tubing were removed from anaesthetized animals one week after implantation.

2. 2. 4. Behavioral Training

Animals were placed into one of three training groups: no training (NT), structured training (ST), and functional training (FT). Animals who were in the NT groups stayed in their home cages, and were handled when cages were cleaned or during testing. ST consisted of daily skilled reaching training for one hour on the Whishaw reaching task, 5 days per week over a period of four months. This type of training was considered a structured one, because there were specific muscle groups being utilized for reaching. FT consisted of rats living 24 hours per day in the complex cage housing environment. This environment encouraged the use of all muscle groups through the ability to move in an enlarged area with additional stimuli to encourage interaction. During training and testing some animals that were in the ST and FT groups were given bracelets to prevent reaching with the ipsilateral or non-dominant paw if necessary. These bracelets prevented the animal from using the non-dominant paw in reaching through the bars to retrieve food. A strip of elastic plaster, Elastoplast (Smith and Nephew, Lachine, Quebec, Canada) 2 cm wide and 6 cm long was used. At one end, the plaster was folded side ways so that the sticky sides faced each other. The remaining section, then, had an adhesive surface. The elastic was wrapped around a rat's forearm and fixed with the exposed plaster. Use of the bracelets in this way prevented denuding the hair on the rat's forearm. Once the rats were habituated to the bracelets they usually ignored them. After habituation, most of the rats learned to use their dominant limb even when the bracelets were not present (Whishaw, 2000).

2. 2. 5. Testing

Testing took place every 14-18 days over four months for all trained and untrained groups totaling five test sessions in all. Animals were food-deprived prior to testing. Rats were videotaped for all tests, and behavioral analyses were performed subsequent to the videotaping. The battery of tests included 1) a skilled reaching test for 5 minutes, 2) a spontaneous vertical exploration test for 5 minutes, 3) forepaw use during swimming, 4) single pellet reaching, and 5) tongue extension. Brain weights, cortical thickness, and claw cutting abilities were examined after the rats were sacrificed.

2. 2. 5. 1. Skilled Reaching

This test was the same as the skilled reaching task described previously. Scoring consisted of taking the total number of successful hits divided by the total number of hits and reaches x 100 for each paw to establish a percentage. The paw with the highest percentage of hits was considered the dominant paw.

2. 2. 5. 2. Spontaneous Vertical Exploration

This test is sensitive to chronic limb use asymmetries (Schallert & Lindner, 1990; Jones and Schallert, 1994; and Liu, et al., 1999). As described in Liu et al., animals were singularly placed in a clear plexiglas cylinder (30 cm in diameter and 45 cm high) for 5 minutes. Rats freely explored the space by using their forelimbs on the cylinder wall (Figure 2. 3). Control animals tend to use one paw or both paws approximately 50% of the time; whereas unilateral lesion

animals tend to use the paw ipsilateral to the lesion or the non-dominant paw. Vanilla extract or chocolate chip mash was dabbed around the top of the cylinder to motivate the animals to explore the wall. The cylinder was placed on a glass table. A mirror, angled at 45° was positioned below the glass. In this way, the forelimbs could be viewed at all times. The testing session was videotaped and scored at a later date

Figure 2. 3. Spontaneous vertical exploration task.

Two behaviors were scored: 1) independent use of the left or right forelimb in contacting the cylinder wall during a rear, initiation of a weight-shifting movement, or in moving laterally along the wall in a vertical position; and 2) simultaneous (within 0.5 seconds of each other) use of both the left and right forelimbs during a rear or to move laterally along the wall. For example, if the dominant paw was placed on the wall, followed by a delayed contact, the animal would receive a score of one "dominant" and one "both" for that sequence. If

only one forelimb contacted the wall and continued lifting and re-contacting the wall, all subsequent movements after the first one would be scored as independent movements. If the rat explored the wall by using both forelimbs, then alternated movements with both limbs (wall stepping), the combination of the two-limb movements would be scored as "both." Scoring consisted of establishing a percentage of non-dominant paw contacts divided by the total number of dominant, non-dominant, and bilateral wall contacts x 100 to establish a percentage.

2. 2. 5. 3. Forepaw Use During Swimming

When swimming forward in water, normal rats tend to hold both forepaws under their chin, keeping the paws immobile and using their hindlimbs to propel themselves (Schapiro, Salas, & Vukovich, 1970, [Figure 2.4.A]). In unilateral lesion rats, only the unaffected forelimb tends to remain immobile, while the affected forelimb produces strokes along with the hindlimbs (Stoltz, Humm, & Schallert, 1999, [Figure 2.4.B]). Rats were tested in a rectangular aquarium (90 cm x 43 cm x 50 cm) with methods similar to Stoltz et al. (Figure 2.4.C). A visible 26 cm high and 9 cm squared wire mesh platform was at one end of the aquarium in water 25 cm deep and approximately 22° C. Rats were initially trained to orient to the task by being placed into the water at progressively longer distances from the platform until they were able to swim directly to it when released from the opposite end of the aquarium. Until the rats learned the protocol, they would tend not to inhibit the forelimb movements until they could

swim directly to the platform. After the animals completed five successful trials, they were given pieces of chocolate chip cookies or food pellets and placed under a heat lamp as reinforcement. A swim score was quantified by summing the number of strokes made with the impaired forelimb minus the number of strokes made with the unimpaired forelimb as a mean of all 5 trials. This was considered the forepaw inhibition index.

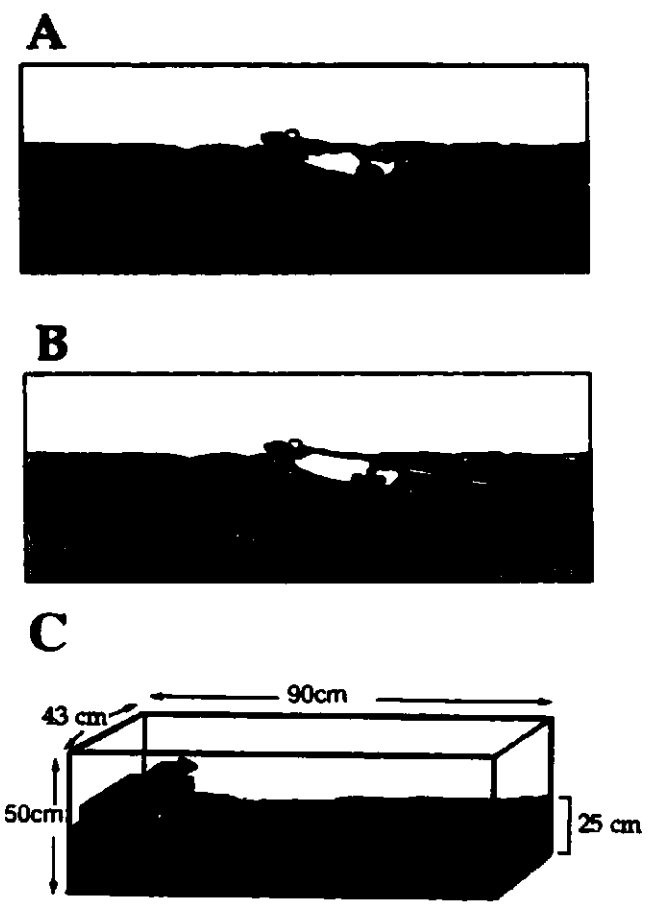


Figure 2. 4. A. Normal immoblile forepaw position when swimming forward.

2. 4. B. In unilateral lesion animals, the affected forelimb produces strokes.

2. 4. C. Testing apparatus to examine forepaw use during swimming.

(After Stolz, Humm, and Schallert, 1999).

2. 2. 5. 4. Tongue Extension

Tongue protrusion in rats can be examined easily to determine whether feeding abnormalities are present in lesion animals (Whishaw & Kolb, 1983). It appears that the orbital frontal cortex and corticofugal pathways adjacent to the lateral hypothalamus are implicated in control of tongue and mouth use. Control animals tend to have tongue extension lengths of approximately 10-12 mm, whereas lesion animals with damaged cortical areas affecting tongue and mouth use, tend to have tongue extension lengths of 1-6 mm. To examine tongue extension, a spatula was covered with chocolate chip cookie mash made with chocolate chip cookies and water. The spatula was then placed flush to the front of the home cage in a vertical fashion, where each animal was tested individually. Tongue extension length was obtained by having the animals remove the cookie mash by licking it off the spatula. The spatula was placed in an area of the front cage where it would be easiest for the animals to reach. The area that the animal licked was then measured. Average tongue extension length was established for each animal over the five test sessions.

2. 2. 5. 5. Single Pellet Reaching

Rats were tested in single pellet reaching boxes to examine qualitative features of the actual reaching movements similar to procedures described in Whishaw, Pellis, Gorny, Kolb, & Tetzlaff, 1993; Whishaw, 2000; Metz & Whishaw, 2000). Boxes were made of clear Plexiglas 11 x 38 x 40 cm high. In the exact center of the front wall was a 1.5 cm wide slit that extended from the

floor to a height of 31 cm. On the outside of the wall, in front of the slit, mounted 4 cm above the floor, was a 3 cm wide by 10.5 cm long shelf. Two small indentations, each .5 cm in diameter, were located on the floor of the shelf. These held food pellets (45 mg Rodent Chow food pellets, Bioserve Inc., P.O. Box 250, Frenchtown, NJ). The indentations were 2 cm away from the inside wall of the box and were positioned on the outer edges of the slot (Figure 2. 5). Food was placed in the indentation contralateral to the dominant paw for each rat. Training was accomplished by having animals successfully reach for and receive a pellet, followed by placing a food pellet in the back of the box. This encouraged the rat to move from the front opening and reposition itself prior to the next food pellet.

Three successful reaches for each rat were rated for qualitative features of the movement. These movements included: 1) *Orient*, the head is oriented to the target food and sniffing occurs; 2) *Limb lift*, the body weight is shifted to the back, the limb is raised from the floor with the upper arm, and digits are moving to the midline; 3) *Digits close*, the digits are semi-flexed and the paw is supinated with the palm facing toward midline; 4) *Aim*, the upper arm raises the elbow, adducting it so that the forearm is midline with the body and the paw is under the mouth; 5) *Advance*, the head is lifted, the elbow is adducted, and the limb is directed to the target as the body weight shifts forward and laterally; 6) *Digits open*, the digits are extended as the limb moves forward; 7) *Pronation*, the upper arm moves, abducting the elbow and the paw moves directly over the food with the palm down; 8) *Grasp*, the arm is still as the digits close onto the food,

then the paw lifts; 9) *Supination I*, the limb withdraws, the elbow is adducted, and the paw is supinated 90°, facing medially before leaving the slot; 10) *Supination II*, the head is pointed downward, the paw is supinated another 90°, as food is presented to the mouth; and 11) *Release*, the digits are opened and food placed into the mouth.

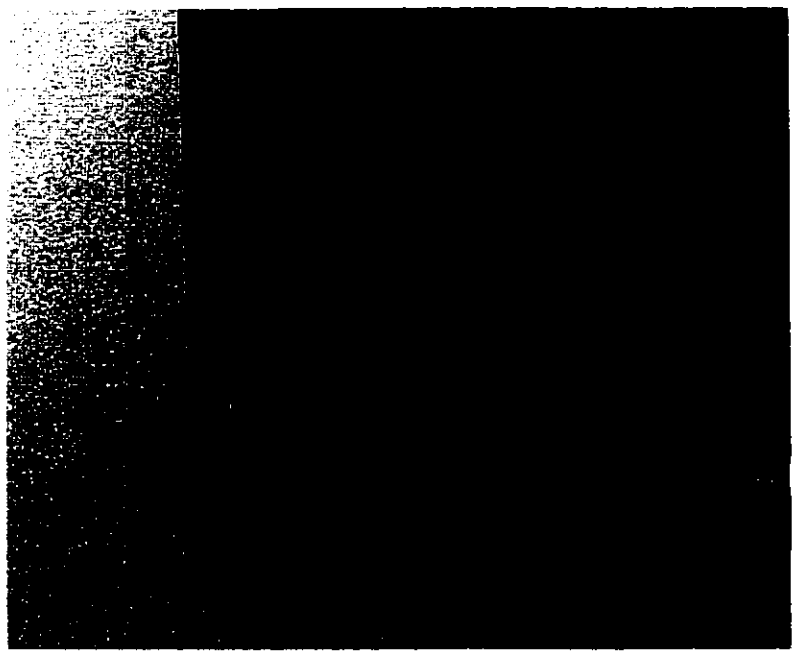


Figure 2. 5. Single pellet reaching box.

Each of the movements was rated on a 3-point scale. If the movement appeared normal, it was given a score of "0"; if the movement appeared slightly abnormal but was recognizable, it was given a score of "1"; and if the movement was absent or compensated entirely by movement of a different body part, a score of "2" was given. Animals were videotaped until three successful reaches were made and scores were established for each of the 11 reaching components. A successful reach was one in which the animal reached for and

ate the pellet on the first attempt. An independent scorer rated the animals.

2. 2. 5. 6. Claw Cutting

Examination of claw length can provide information about an animal's behavioral competencies (Figure 2. 6). Loss of the ability to claw cut appears to be due to inefficient biting and chewing as opposed to grooming (Whishaw, Kolb, Sutherland, & Becker, 1983). Claws were examined according to procedures established by Whishaw et al. (1983). When the rats were sacrificed for brain histology, the length of all the rear paw claws was measured to the nearest .5 mm. The measure was made from the cuticle (tissue surrounding the proximal edge of the claw) to the tip. Mean claw length for each paw was recorded.

Figure 2. 6. Photograph of a rear paw of a control rat (left) and a rat having a cortical lesion (right). Note the shorter length of the nail on the control rat and the longer or broken nails on the lesion rat (From Whishaw et al., 1983, p. 372).

2. 2. 5. 7. Preparation of Brains

After all testing was completed, rats were deeply anesthetized with .5 cc of euthanol, and intracardially perfused with a solution of 0.9% physiological saline. The brains were extracted whole and weighed. In order to weigh the brains, the olfactory bulbs were blocked off 2 mm from the tip of the cerebral hemispheres, and the cerebellar flocculi and pineal gland removed. Brains were then placed into 20 ml of Golgi-Cox solution (Glaser & van der Loos, 1981). Brains remained in this solution for 14 days, then were placed into 30% sucrose for at least three days prior to being sectioned. Brains were photographed, then blocked perpendicular to the midline at approximately the level of the anterior commissure and again through the caudal portion of the occipital cortices. Coronal sections were cut in 200 µm on a vibratome into 6% sucrose solution, then mounted on 2% gelatin-coated slides, and developed according to methods by Kolb and McLimans (1986). Cortical thickness was measured according to methods described by Stewart and Kolb (1988). Golgi-Cox stained sections were projected on a Zeiss DL 2 POL petrographic projector set at a magnification of 10x. Measurements made with a plastic millimeter ruler were taken at three points in each of five planes (Figure 2.7). All measurements were made without knowledge of the group identity of the animals by an independent examiner.

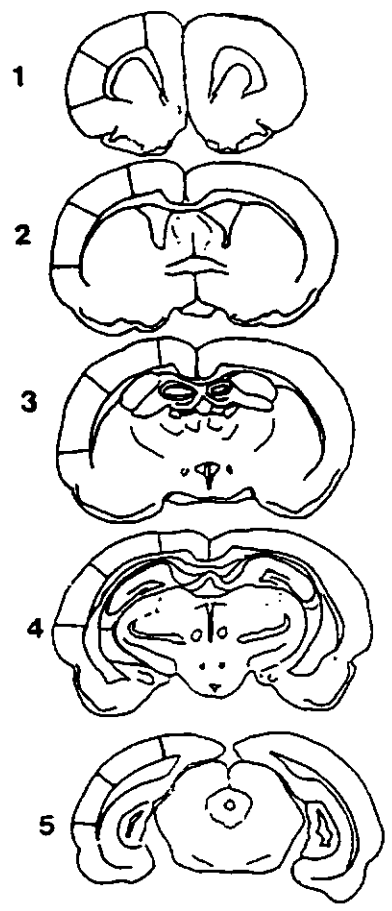


Figure 2. 7. Coronal sections through the rat brain at which measurements were taken. Three measurements were taken in each hemisphere as indicated by the lines. Lateral, medial, and central areas respectively are described following the names of the cortical areas according to Zilles (1985). Plane 1: First plane with caudate-putamen visible (Gu, Par 1, Fr 2). Plane 2: Anterior commissure (Par 2, Par 1, Fr 1). Plane 3: First hippocampal section (Gu, Par 1, Fr 1). Plane 4: Posterior commissure (Te 1, Oc 2L, RSA). Plane 5: Most posterior hippocampal section (Te 1, Oc 1B, Oc 2ML). (From Stewart & Kolb, 1988, p. 348).

2. 2. 5. 8. Statistical Methods

We were interested in two primary measures: 1) improvement over time as assessed by repeated measures analyses of variance (ANOVAs); and improvement at the end of training as assessed by doing a two-way ANOVA with lesion group and training as factors on test day 5. In other words, results were summed across all data, summed across time, and summed across day 5. Fisher's PLSD was used for post hoc evaluations.

2. 3. RESULTS

2. 3. 1. Skilled Reaching

All rats with motor cortex lesions were impaired at reaching for food. Some NT animals reverted to their non-dominant paw. Animals in the ST groups tended to stop reaching after approximately 30-45 minutes. However, FT lesion rats performed as well as NT animals, and trained bFGF rats, both in ST and FT groups performed as well as the NT controls. Structured behavioral training improved scores in all groups compared to the NT groups. A repeated measures ANOVA with lesion group, training, and test days as factors revealed a significant main effect of group ($F(2,45)=13.506$, $p< .0001$), and training ($F(2,45)=6.87$, $p= .0025$), but not test day ($F(4,180)= .467$, $p= .760$) (Figure 2. 8). The only significant interaction was time x treatment ($F(8,180)=2.59$, $p= .011$). A two-way ANOVA on test day 5 with lesion group and training as factors showed a significant main effect of group ($F(2,45)=9.943$, p-.0003), and training ($F(2,45)=5.852$, $p= .0055$), but no interaction

(F (4,45)=1.148,p= .3465) (Figure 2. 9). As seen in Figure 2.8, then, three distinct groupings appeared by test day five performing from best to worst, respectively: 1) CT animals, 2) NT controls, FT lesion, FT bFGF, and FT lesion animals, and 3) untreated lesion, untreated bFGF, and ST lesion animals.

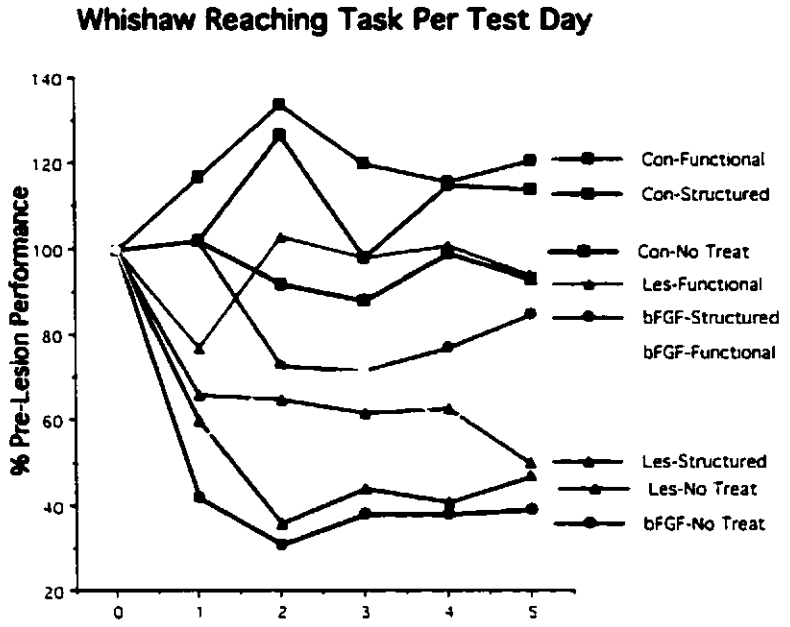


Figure 2. 8. Repeated measures on the Whishaw reaching task over five test days, comparing scores to pre-lesion performance. Note that three distinct groupings occur on test day five.

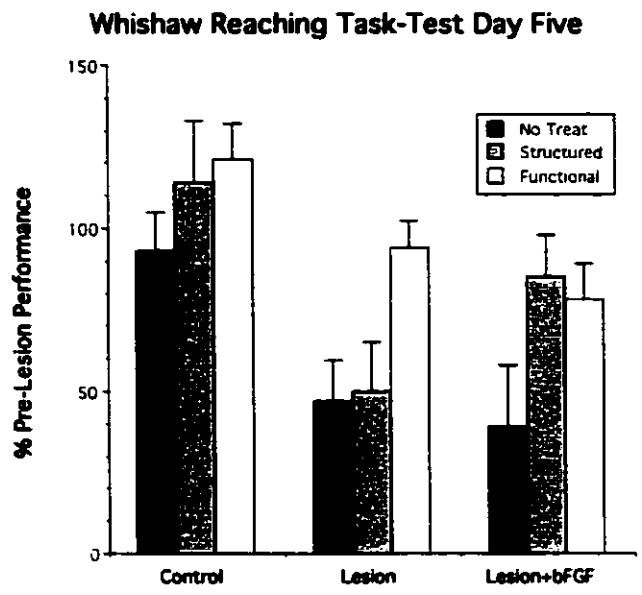


Figure 2. 9. Whishaw reaching task-test day five. There is an overall effect of group and treatment, with functional lesion, structured bFGF, and functional bFGF groups performing as well as untrained controls.

2. 3. 2. Spontaneous Vertical Exploration

Initially, animals actively explored the plexiglas cylinder. As testing proceeded during the ensuing weeks, animals tended to become habituated to the environment within the cylinder and exploration decreased. They were more motivated to explore if they were food-deprived, and the scent of vanilla extract or chocolate chip cookie mash was near the top of the cylinder. Several of the FT animals even jumped to the rim of the cylinder. Over time, all trained groups decreased the use of their non-dominant paw relative to untrained groups, indicating an improvement in function of the affected paw. In other words, with training, animals were more likely to use their affected limb, or to increase the

use of bilateral movements. A repeated measures ANOVA with lesion group, training, and test days as factors revealed a significant effect of group ($F(2, 46)=15.83, p< .0001$), and training ($F(2, 46)= 3.507, p= .0382$), but not test day ($F(4, 184) =1.625, p = .1696$). A two-way ANOVA on test day five with lesion group and training as factors showed an effect of group ($F(2,46)=5.498, p= .0072$), and training ($F(2,46)=3.287, p= .0463$), but no interaction ($F(4, 46) = .967, p= .4347$) (Figure 2.10). The treatment effect was due to enrichment, comparing the functional and non-treated groups, so animals in the FT groups increased the use of their dominant paw (Figure 2.11).

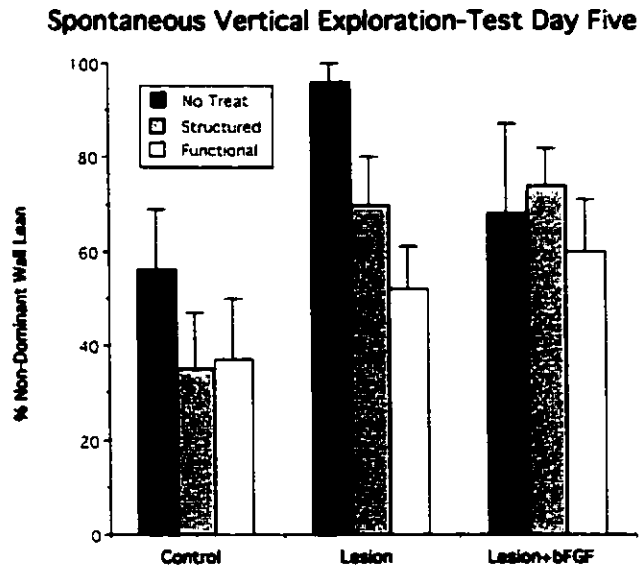


Figure 2. 10. Percent non-dominant wall lean on test day five during the Schallert spontaneous vertical exploration task. Functional lesion and bFGF groups performed as well as the untrained controls.

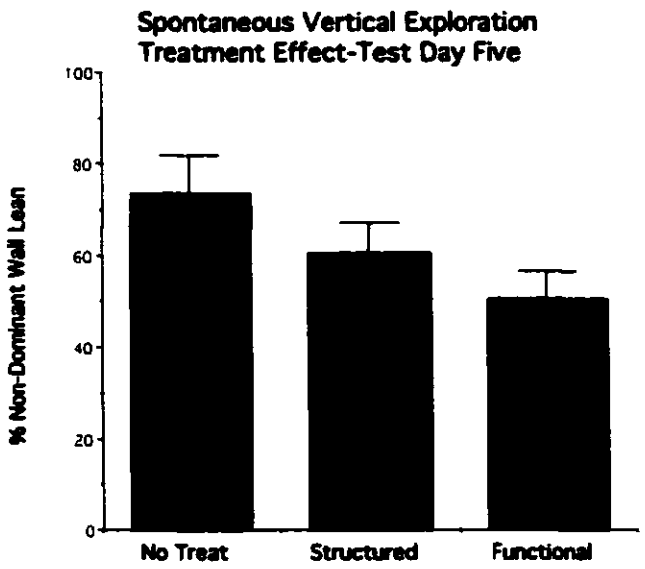


Figure 2. 11. Percent non-dominant wall lean on test day five during the Schallert spontaneous vertical exploration task. There is an enrichment effect comparing non-treated and functional groups.

2. 3. 3. Forepaw Use During Swimming

After rats were trained to swim directly to the platform, control animals tended to hold both forelimbs motionless underneath the chin, whereas lesion animals often used the impaired dominant limb to stroke. A repeated measures ANOVA with lesion group, training, and test days as factors revealed a significant effect of group ($F(2,46) = 13.28, p < .0001$), no effect of training ($F(2,46) = .003$, $p = .9969$), but an effect of test day ($F(4,184) = 2.377, p = .0536$). The major effect of test day was mainly due to improvements in bFGF groups. In a parallel study, however, we have shown that there is a trend toward recovery after one year in

lesion animals, which suggests that the processes related to recovery may be more protracted than expected. A two-factor ANOVA on test day five with lesion group and training as factors revealed a group effect (F (2,46) =8.72,p= .0006), but no training effect (F (2,46) =.16,p= .8541), or interaction, (F (4, 46) =.54,p = .7101) (Figure 2.12). Post-hoc testing, however, indicated a significant effect comparing control and lesion, control and bFGF, and lesion and bFGF groups (Figure 2. 13). The bFGF groups, then, used forepaw immobility in swimming more than those in the lesion groups.

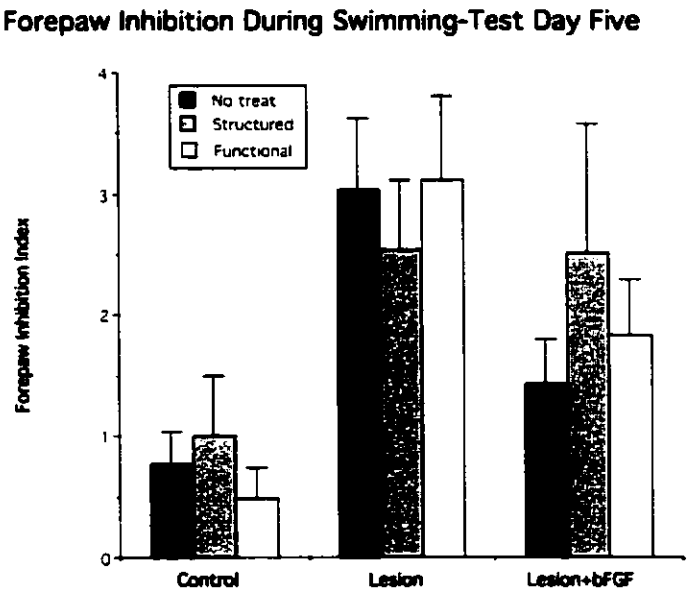


Figure 2. 12. Forepaw inhibition index on test day five. Note that bFGF NT and FT groups performed best out of all lesion groups.

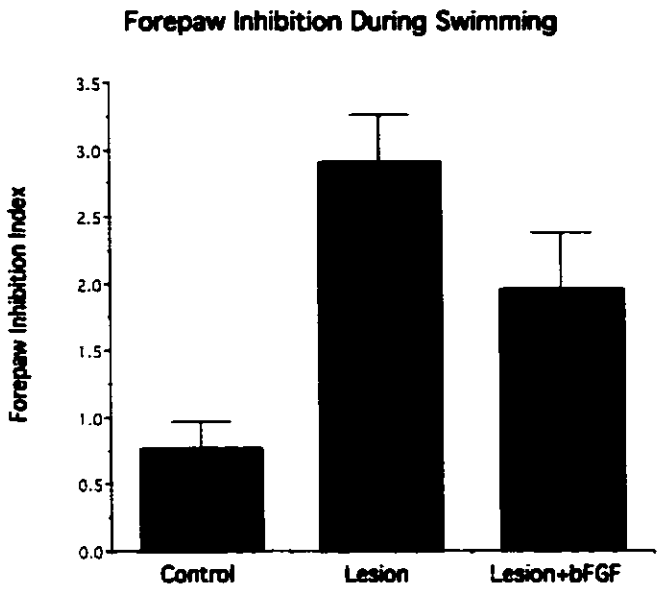


Figure 2. 13. Group effect of forepaw inhibition during swimming on test day five.

2. 3. 4. Tongue Extension

Animals in all groups had tongue extension lengths of 9-13 mm, indicating the absence of damage in the orbital frontal cortex, an area controlling tongue movements. ANOVA of mean tongue extension length collapsed over five test days indicated no effect of group ($F(2,45) = .024, p = .9763$), no effect of training ($F(2,45) = 1.82, p = .1731$), but an interaction ($F(4,45) = 5.42, p = .0012$). This interaction had to do with the FT bFGF group having significantly longer tongue extension lengths than all other groups. Repeated measures ANOVA revealed an effect of time ($F(4,160) = 9.822, p < .0001$). Follow-up repeated measures ANOVA examining lesion and bFGF separately showed an effect of treatment in the lesion group where the FT group had smaller tongue extensions than the

others, and in the bFGF group there was an effect of treatment where the FT group had longer tongue extensions than the others. Table 2. 1 summarizes tongue extension lengths collapsed over 5 test sessions.

Table 2. 1. Summary of Tongue Extension.

Group	No Treat	Structured	Functional
Control	11.0 ± .17	10.5 ± .14	11.4 ± .20
Lesion	11.1 ± .17	11.2 ± .20	10.5 ± .14 [a]
bFGF	10.6 ± .20	10.4 ± .18	11.8 ± .20 [b]

Note: Numbers refer to means and standard errors. Numbers represent length in mm.

[a] Differs significantly from the other lesion groups, p=.0594.

[b] Differs significantly from the other bFGF groups, p=.0127.

2. 3. 5. Single Pellet Reaching

Qualitative features of reaching were examined with this test to determine whether experience, lesion, and/or bFGF altered the way the animals achieved food. Despite the fact that animals were pre-trained to reach in the reaching cages, this task was new to them, and they also needed some training to this environment prior to successful retrieval of pellets on the first reach. Some animals were unable to successfully use their dominant paw, and they were excluded from the analysis.

An analysis of the data was accomplished by examining each of the 11 components according to lesion group. A one-factor ANOVA was done comparing the ST or FT group with the NT group for each component. In Table 2.2 is listed those components that varied significantly from the NT group, either positively or negatively. For example, in the first component "orient," the FT controls did significantly better than the NT controls, etc. In observing the table, "orient" and "limb lift" were affected in the FT controls, "orient" and "digits close" were affected in the ST lesion group, and "supination II" and "release" were affected in the ST and FT bFGF groups. In terms of the FT controls, it appeared that the way the animals approached the food varied. In terms of the lesion groups, one would expect that those animals experiencing daily structured reaching would perform better than the NT animals on these tasks. Rather, in the ST lesion group, the abilities of the animals in orienting to the food and in preparing the paw and digits to aim for the food were negatively affected. In terms of the bFGF groups, both the experience of the structured reaching activity and the functional enriched environment positively affected the quality of the components of "supination II" and "release." An additional analysis comparing ST and FT in the lesion group indicated the following components were significantly affected on post hoc testing in favor of FT: "orient" ($p<.0001$), digits close ($p=.0476$), and supination I ($p=.0203$).

Overall, this test indicated positive effects of bFGF and FT training in the qualitative movements of reaching, particularly in the component areas of "orient," "digits close," "supination I," "supination II" and "release".

Table 2.2. Summary of Single Pellet Reaching

Component	Control		Lesion		bFGF	
	Structured	Functional	Structured	Functional	Structured	Functional
1. Orient		+	--			
2. Limb lift		--				
3. Digits close			--			
4. Aim						
5. Advance						
6. Digits open						
7. Pronation						
8. Grasp						
9. Supination I						
10.Supination II					+	+
11. Release					+	+

Note: + indicates better performance compared to the NT groups.

-- indicates worse performance compared to the NT groups.

2. 3. 6. Claw Cutting

After animals were perfused, hindpaw nail length was measured to examine chewing and biting abilities. Mean nail length was calculated for each group (Table 2. 3). A two-factor ANOVA revealed no effect of group ($F(2,43) = .68, p = .5143$), no effect of training ($F(2,43) = 1.65, p = .2049$), but an interaction ($F(4, 43) = 5.88, p = .0007$). The interaction was due to the effects of bFGF. ANOVA on the bFGF groups indicated a training effect ($F(2,14) = 19.80, p < .0001$). Post-hoc evaluation showed significant differences between FT, ST, and NT groups (Figure 2.14).

Table 2. 3. Summary of claw cutting.

Group	No Treat	Structured	Functional
Control	4.3 ± .21	4.3 ± .28	4.7 ± .05
Lesion	4.4 ± .27	4.2 ± .10	4.5 ± .08
bFGF	4.9 ± .06[a]	4.3 ± .16[b]	3.7 ± .13[c]

Note: Numbers refer to mean nail length in mm and standard errors.

[a] Differs significantly from [b] and [c], and [b] differs significantly from [c].

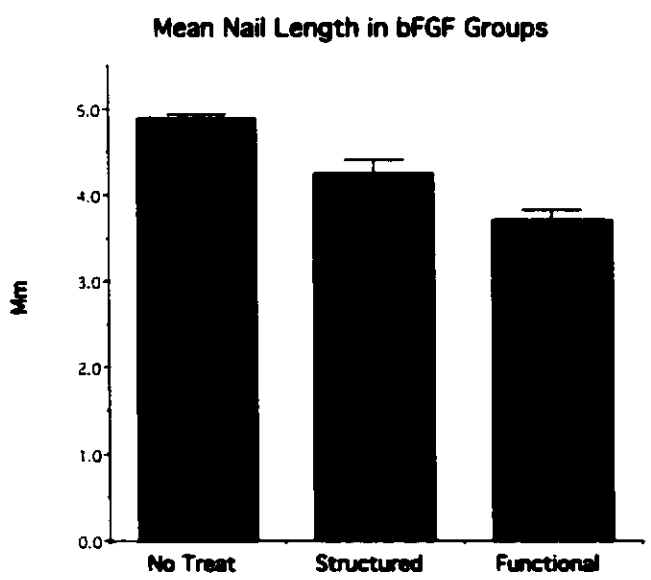


Figure 2. 14. Mean nail length in bFGF groups. The FT group has the shortest nail lengths, followed by the ST group, implying better biting and chewing abilities.

2. 3. 7. Brain Weight

Figure 2.15 shows representative examples of a control, lesion and bFGF brain from a dorsal view. A two-way ANOVA examining brain weights with lesion group and training as factors showed no group effect ($F(2,43) = .35, p = .7073$), no training effect ($F(2,43) = 1.37, p = .2645$), but an interaction ($F(4,43) = 2.93), p = .0314$). A one-way ANOVA was then done to examine effects of treatment in each group. There was an effect of treatment in the control group ($F(2,14) = 3.39, p = .0517$), and in the bFGF group ($F(2,14) = 3.73, p = .0503$), but not in the lesion group ($F(2,15) = .21, p = .8154$). The control NT and the bFGF FT groups had the largest brain weights (Table 2.4).

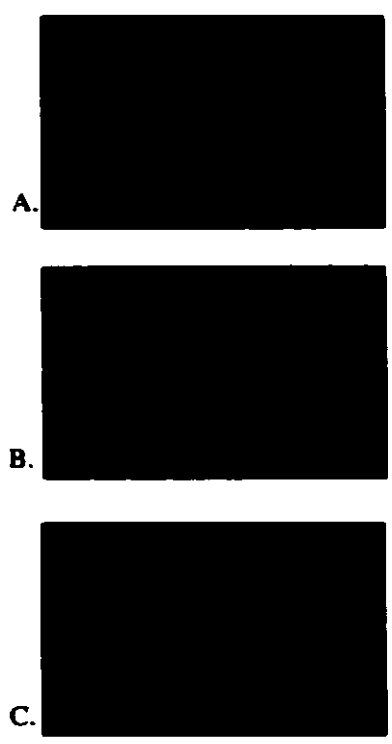


Figure 2. 15. Representative examples of control (A), lesion (B), and bFGF (C) brains.

Table 2.4. Summary of Brain Weights.

Group	No Treat	Structured	Functional
Control	$2.20 \pm .017^{a}$	$2.11 \pm .026^{b}$	$2.13 \pm .033$
Lesion	$2.12 \pm .038$	$2.15 \pm .035$	$2.14 \pm .026$
bFGF	$2.08 \pm .047^{d}$	$2.09 \pm .007^{d}$	$2.21 \pm .049^{c}$

Note: Numbers refer to mean brain weights in grams and standard errors.

[a] differs significantly from [b] and [c] differs significantly from [d].

2. 3. 8. Cortical Thickness

Observations of coronal sections revealed that the lesions removed the intended targets. Cortical thickness was analyzed according to lesion (dominant) or intact (non-dominant) hemisphere. Cortical thickness in control animals was determined by using the hemisphere contralateral to the dominant paw. Overall, the lesion and bFGF groups had thinner cortices in the lesion hemisphere. Figure 2.16 shows serial drawings of Golgi-Cox stained-coronal sections through the brain of a representative unilateral lesion rat.

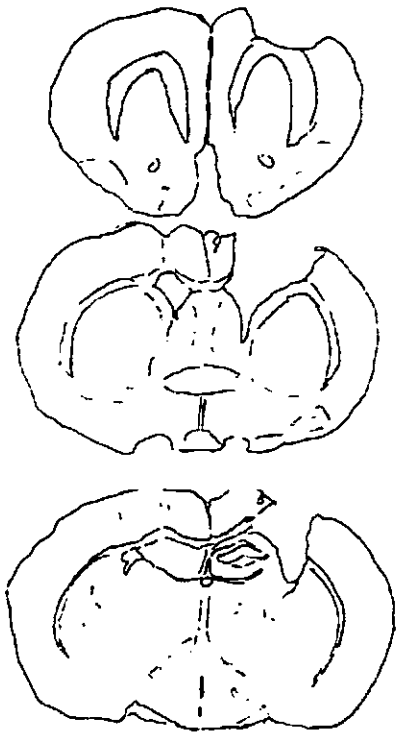


Figure 2. 16. Serial drawing of Golgi-Cox stained-coronal sections through the brain of a representative unilateral motor cortex lesion rat in planes 1-3.

A two-way ANOVA was performed on the mean cortical thickness measurements averaged across all planes with lesion group and training as factors. In the lesion hemisphere there was a group effect ($F(2, 37) = 12.44, p < .0001$), but no training effect ($F(2, 37) = .27, p = .7643$), or interaction ($F(4, 37) = 2.22, p = .0856$) (Figure 2.17A). Interestingly, the ANOVA for the intact hemisphere showed no group effect ($F(2, 37) = .50, p = .6099$), no treatment effect ($F(2, 37) = 1.01, p = .3746$), but an interaction ($F(4, 37) = 3.26, p = .017$) (Figure 2.17B). The interaction had

to do with a larger cortical thickness in plane 1 in the bFGF groups, mainly in the FT group.

A repeated measures ANOVA across the five planes was conducted on each hemisphere. For the lesion side, both the main effects of lesion group ($F(2, 43) = 11.91, p < .0001$), and planes ($F(4, 172) = 64.2, p < .0001$), were significant as was the interaction ($F(8, 172) = 3.99, p = .0002$). For the intact side, there was no effect of group ($F(2,43) = .60, p = .553$), but there was an effect of the plane factor ($F(4,172) = 142.22, p < .0001$), then a trend toward an interaction ($F(8,172) = 1.94, p = .0577$).

Finally, one-way ANOVAs were done on each plane for each hemisphere. On the lesion side, both the lesion and bFGF groups had significantly thinner cortices than controls in planes 1 and 2. Post hoc testing showed a significantly thinner cortex in the NT groups compared to the ST groups mainly due to the thinner cortex in the NT bFGF group in plane 3. On the intact side, the only significant difference was in the bFGF FT group having a larger cortical thickness than controls, and the lesion ST group having a smaller cortical thickness than the corresponding FT and NT groups in plane 1. In summary, lesion and bFGF groups had overall thinner cortices in the lesion hemisphere, whereas in the intact hemisphere, there was a thicker cortex in the bFGF FT group.

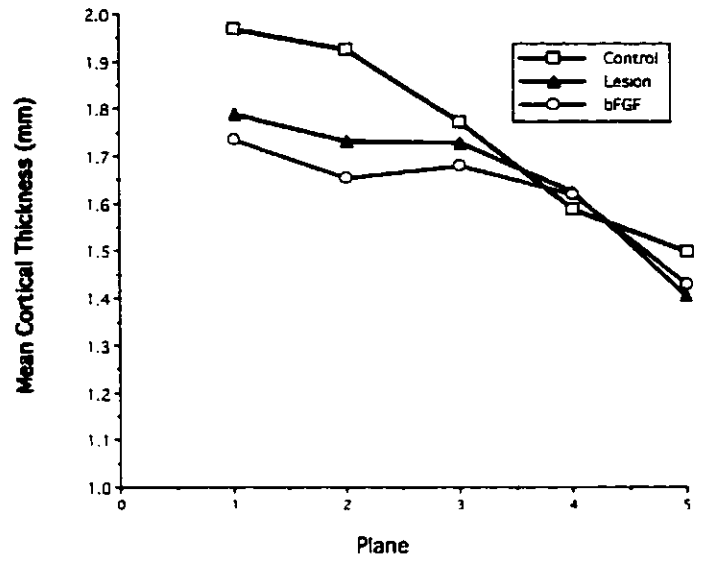


Figure 2. 17. A. Mean cortical thickness in the lesion hemisphere in each of five planes.

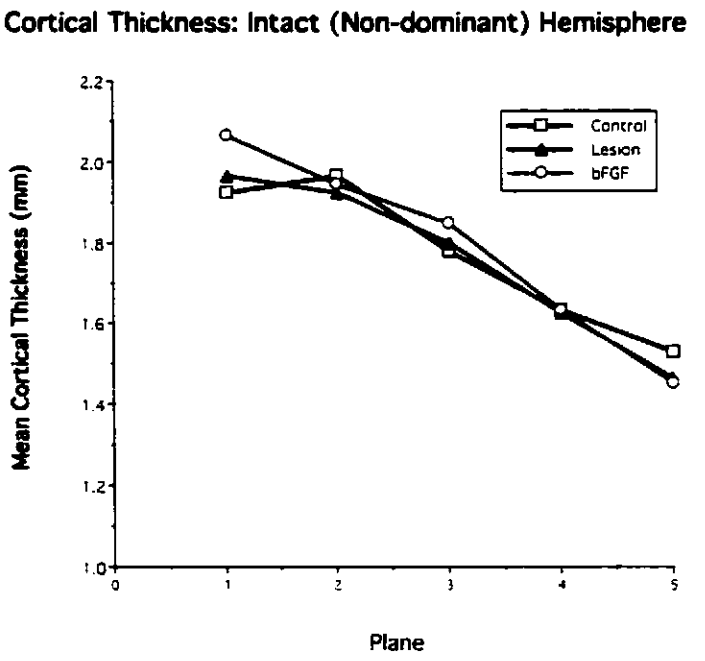


Figure 2. 17. B. Mean cortical thickness in the intact hemisphere in each of five planes.

2. 4. DISCUSSION

This experiment compares structured training (skilled reaching) and functional training (enriched environment) with and without postlesion infusion of FGF-2 in rats having unilateral motor cortex lesions. Table 2.5 synopsizes the results of testing in this experiment by listing the battery of tests administered and indicating whether there were significant changes (+) across groups.

Table 2. 5. Comparison on a battery of tests between training/treatment groups. Significantly improved results across groups are represented by (+).

TEST ADMINISTERED	ST	FT	ST+ bFGF	FT+ bFGF	NT+bFGF
Whishaw Reaching Task	--	+	+	+	--
Spontaneous Vertical Exploration Task	--	+	--	+	--
Forepaw Inhibition During Swimming	--	--	--	+	+
Tongue Extension	--	--	--	+	--
Single Pellet Reaching	--	--	+	+	--
Claw Cutting	--	--	--	+	--
Brain Weight	--	--	--	+	--
Cortical Thickness: Lesion (Dominant) Hemisphere	--	--	--	--	--
Cortical Thickness: Intact (Non-Dominant) Hemisphere	--	--	--	+	--

Overall, there were five important findings from this study: 1) There is a difference in rehabilitation-induced recovery of function between structured training and functional training paradigms; 2) FGF-2 does enhance functional recovery, but mainly with accompanying physical structured or functional training; 3) Recovery can improve over time and be maintained at least up to four months; 4) The combination of functional training and FGF-2 treatment improves rehabilitation-induced recovery of function more than either treatment alone; and 5) The degree of recovery of function is best examined using a battery of tests to differentiate compensation versus true recovery. Each finding will be discussed in turn.

2. 4. 1. ST versus FT and rehabilitation-induced recovery of function

Structured daily training alone over four months did not significantly improve rehabilitation-induced recovery of function on any subtest, unless FGF-2 was an additional treatment. With functional training alone, more accurate use of the affected paw in skilled reaching occurred, as well as a decrease in relying on the non-affected paw for vertical exploration. However, because there was no significant treatment effect noted in the single pellet task in which the qualitative features of reaching were examined, this implied that the improvement in skilled reaching was compensatory in nature. That is, both skilled reaching and single pellet reaching tasks require fine motor movements of the wrist and digits. However, in the Whishaw reaching task, the movements are rated as a percentage of success in reaching for food, and different compensatory

strategies are used to accomplish this reachng successfully but are not specifically scored. In the single pellet reaching task, on the other hand, the individual movements are rated on an 11 point scale, and so closer observation and scoring is done with the individual wrist and digit movements.

2. 4. 2. FGF-2 enhances rehabilitation-induced recovery of function

With the additional treatment of FGF-2, the ability for rehabilitation-induced recovery improved as shown in overall test results. There were some significant improvements noted in the ST+FGF-2 group in skilled reaching and single pellet reaching, indicating an improvement in the qualitative aspects of reaching. Again, the combination of FT and FGF-2 improved functional recovery in every behavioral and anatomical measure, except for cortical thickness in the lesion hemisphere. Interestingly, the lesion group having FGF-2 and no treatment only improved significantly on one subtest: forepaw inhibition during swimming. Results in the other subtests were similar to the ST group. In other words, little recovery of function was found with the treatment of FGF-2 alone.

2. 4. 3. Recovery of function over time and maintained improvement

In performing repeated measure ANOVAs, improvements were noted over time on many of the subtests (i.e., Whishaw reaching task, tongue extension, single pellet reaching, and claw cutting). Results were maintained over the four month period as shown by ANOVAs on test day five. Many animals in the non-treated groups reverted to their non-dominant paw, progressively decreased their

performance success, or made no demonstrable changes on subtest results.

2. 4. 4. FGF-2 and FT combined produced the best functional recovery

As mentioned above, overall test results in ST alone, FT alone, and treatment of FGF-2 alone indicated less improved rehabilitation-induced recovery of function than the synergistic interaction between FGF-2 and FT. The combination of treatments produced remarkable improvement on every subtest. A possible mediating factor could be due to the enhanced plasticity in the intact hemisphere of this group, as evidenced by the significant increase in cortical thickness compared to the other groups.

2. 4. 5. Use of a battery of tests to accurately determine recovery

The best way to determine behavioral degree of recovery or compensation depends on continuous testing over time and with a number of tests that accurately reflect intact versus damaged functions. In view of the hierarchical organization of levels of brain function, several areas of the brain mediate similar functions. It is likely they may do so in slightly different ways, however, and a variety of behavioral tests will be needed to determine which aspect of a skill is present, impaired, or absent. For example, skilled reaching and spontaneous vertical exploration involve use of upper limb motor movements, but the former involves more fine motor accuracy and the latter involves more generalized elbow and shoulder movement. Assessment using a variety of tests, then, provides invaluable information as to overall functional recovery.

3. EXPERIMENT 2: TACTILE STIMULATION AND RECOVERY OF FUNCTION

3. 1. INTRODUCTION

The results of experiment 1 suggest that functional treatment (complex housing) may be more effective than structured treatment (skilled reaching) in stimulating recovery processes after cortical injury. Previous experiments have shown that functional treatments may also stimulate functional recovery after frontal lesions in infants (Kolb et al., 1998b; Gibb, 2001). Thus, both complex housing from weaning until adulthood as well as tactile stimulation in the days following a neonatal medial frontal lesion on postnatal day 1-5 can stimulate functional recovery in adulthood (for a review, see Kolb, Forgie, Gibb, Gorny, & Rowntree, 1998a; Gibb, 2001). The goal of the current experiment was to determine if a similar treatment might enhance recovery from motor cortex injury in infancy. Infant rats were therefore given motor cortex lesions on postnatal day 4 and received tactile stimulation daily for the next 18 days.

Previous studies have shown that rats with bilateral motor cortex lesions on postnatal day 4 show a poor functional outcome on reaching tests and, in addition, they have unexpected deficits on a test of spatial learning (Kolb, Cioe & Whishaw, 2000a). Because it was possible that the tactile stimulation treatment could be behavior specific, rats were given both the Whishaw reaching task to examine motor abilities, and the Morris water task (Morris, Garrud, Rawlins, & O'Keefe, 1982) to examine spatial learning abilities in adulthood. In addition, because it had been shown that claw cutting was sensitive to early cortical injury,

this too was measured (Whishaw, et al., 1983). Finally, because early cortical lesions produce an abnormally thin cortex (Kolb & Whishaw, 1989), cortical thickness was measured to determine if there might be an effect of tactile stimulation.

3. 2. METHODS AND PROCEDURES

3. 2. 1. Animals

Two separate litters of rat pups from the Charles River Long-Evans strains were placed into treatment (T) and no treatment (NT) groups. In the T group were 12 infants (5 female and 7 male), and in the NT group were 14 infants (6 female and 8 male). The rats were group housed with their respective littermates and the mother in stainless steel hanging cages on a 12 hour light: 12 hour dark schedule throughout the treatment period. They were then weaned and separated from the mother on postnatal day 22 (P 22) and group housed with the same sex littermates. The animals were on ad lib food throughout treatment and testing, except during the food-reaching task when they were food-deprived to 85% of their weight. Animals were cared for under the rules and provisions of the Canadian Council on Animal Care.

3. 2. 2. Surgical Procedures

On P4, pups were randomly given either bilateral motor cortex (T group: 3 F, 4 M; NT group: 3 F, 4 M) or sham (T group: 2 F, 3 M; NT group: 3 F, 4 M) lesions according to methods described by Kolb and Whishaw (1981). Animals were anesthetized by cooling them in a Thermatron cooling chamber set at -5° C

for approximately 15 minutes until their rectal body temperatures were in the range of 18-20° C. The temperature in the cooling chamber was raised to 0° C, and rectal temperature was maintained at this level until surgeries were performed. The entire litter was anesthetized simultaneously. During surgery, the surgeon's fingers held the head. Bilateral motor decortication in Zilles' area FL was achieved by removing the bone over the motor cortex with iris scissors. The neocortex (2 mm anterior to Bregma, 1 mm posterior) was removed by aspiration. Following hemostasis, the scalp wound was sutured with silk thread as soon as the operation was complete. The normal control animals were anesthetized, the skin incised, and then closed with silk suture. After suturing, rats were warmed in cupped hands until movement was noted prior to placing them back with the mother.

3. 2. 3. Behavioral Treatment

Behavioral treatment began the day after surgery and continued until the subjects were weaned. The mode of treatment was tactile stimulation as described in Gibb (2001) and Kolb (1995, p. 151). The T group was removed from their home cage, placed in a holding cage and brushed gently for 15 minutes 3 times each day for 18 days with a 7-mm camel-hair paintbrush (Figure 3.1). They were then returned to the mother in the home cage. The NT group was not handled nor given tactile stimulation during this period.

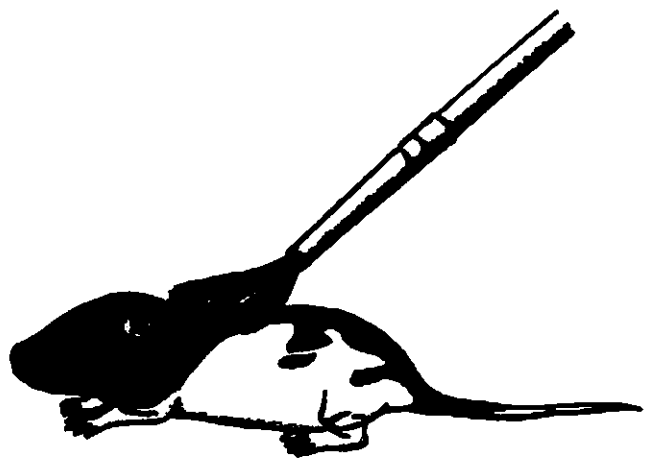


Figure 3. 1. Tactile stimulation. Animals are brushed for 15 minutes, 3 times per day until weaned.

3. 2. 4. Testing

3. 2. 4. 1. Spatial Navigation

On P 60, both litters were tested behaviorally using the Morris water task. The method followed was similar to that used by Sutherland, Whishaw, & Kolb (1983); and Kolb & Gibb (1991). This task was a test of spatial navigation in which rats were placed in a large circular pool of water (diameter 85 cm, height 45 cm). The inside of the pool was painted white and filled to a height of 25 cm with approximately 22° C water, in which one liter of instant powdered skim milk was dissolved to make the water opaque (Figure 3.2). A clear Plexiglas platform (11 cm X 12 cm^2) was present inside the pool with its top surface being one cm below the surface of the water; therefore, the platform was invisible to a viewer inside the pool. The platform was in the southwest quadrant of the pool.

Figure 3. 2. Morris water task.

A trial consisted of placing a rat by hand into the water facing the wall of the pool, at one of the four starting locations, north, east, south, or west, around the pool's perimeter. Each animal completed one block (four trials) per day, the sequence of locations being randomly selected. The water task was conducted on five consecutive days. A trial was terminated if a rat failed to find the platform after 90 seconds. When an animal found the platform within the allotted time, it was permitted to remain there for 10 seconds to orient itself before it was returned to a holding cage to await the next trial. The time between trials for a rat was the time it took for all of the animals in one litter to complete a trial. The latency to find the platform (escape latency) was timed by an experimenter standing by the pool's edge. Escape latency and swimming paths were recorded using a video camera and Poly-Track video tracking system (San Diego Instruments). This system also provided a record of swim latency and swim distance scores.

3. 2. 4. 2. Skilled Reaching

Beginning at P70, rats were trained on the Whishaw reaching task (Whishaw, O'Connor, & Dunnett, 1986; Whishaw, Pellis, Gorny, & Pellis, 1991) to determine forepaw use and paw dominance. Rats were trained, tested, and videotaped in Plexiglas cages similar to that described in Experiment 1 (Figure 2.2).

3. 2. 4. 3. Claw Cutting

Examination of claw length can provide information about an animal's behavioral competencies as evidenced in Figure 2.6 between control and decorticate rats (Whishaw, Kolb, Sutherland, & Becker, 1983). Loss of the ability to claw cut appears to be due to inefficient biting and chewing as opposed to grooming. Claws were examined according to similar procedures described in Experiment 1.

3. 2. 5. Preparation of Brains

At P100, rats were deeply anesthetized with .5 cc of euthanol, and intracardially perfused with a solution of 0.9% physiological saline and 4% paraformaldehyde. In order to weigh the brains, the olfactory bulbs were blocked off 2 mm from the tip of the cerebral hemispheres, and the cerebellar flocculi and pineal gland removed. After the brains were cryoprotected by 30% sucrose, they were photographed, and cut in 40-μm coronal sections in a cryostat set at -20°C. Every fifth section through the lesion area and every tenth section through the rest of the brain was saved, mounted on glass slides, and stained with cresyl violet. Cortical thickness was measured according to methods described by

Stewart & Kolb (1988). Cresyl violet-stained sections were projected on a Zeiss DL 2 POL petrographic projector set at a magnification of 10X. Measurements made with a plastic millimeter ruler were taken at three points in each of five planes (Figure 2. 7). All measurements were made without knowledge of the group identity of the animals by an independent examiner.

3. 2. 6. Statistical Methods

Analyses of variance (ANOVAs) were used for all measures, and Fisher's PLSD was used for post-hoc evaluations. Sex differences were assessed for each measure using a three-way ANOVA, but are only reported when significant.

3. 3. RESULTS

3. 3. 1. Behavior

3. 3. 1. 1. Spatial Navigation

The control rats performed similarly to those described in Sutherland, Whishaw, & Kolb (1983). When initially placed in the milk tank, the normal control rats swam over a wide area until they bumped into the hidden platform. They then climbed up on the platform and reared several times. Performance improved rapidly on successive trials. Rats with lesions also improved with each successive trial, but the initial strategy varied. Generally, the strategy would be to swim around the perimeter of the pool, eventually venture into the middle of the pool, and then find the platform. Because mean escape latency and mean distance traveled yielded similar results, only the latency scores will be reported here. A two-way ANOVA with lesion group and treatment as factors revealed an

effect of group in looking at mean escape latencies (F (1,22)= 6.078,p < .0220), but no effect of treatment (F (1,22)= 1.29,p= .268) nor of the interaction (F (1,22)=2.069,p= .164) (Figure 3.3). Mean total escape latency for the control animals was 25.11 seconds, whereas for the lesion animals it was 36.44 seconds.

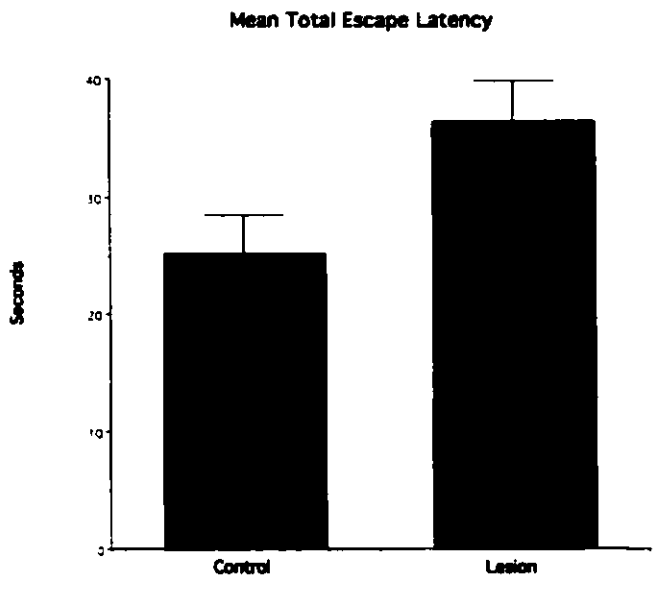


Figure 3. 3. Summary of mean total escape latency with group as a factor.

A repeated measures (trial) ANOVA examining practice effects revealed a significant main effect of trials (F (4, 88)= 84.451, p < .0001) (Figure 3.4). Interestingly, the control NT group performed the best throughout all trials, and there was a significance in the interaction between group and treatment comparing the control T and NT groups, (F (1,25)= 9.077, p < .0064). Figure 3.5

shows mean escape latency per test day, and that learning occurred in both groups over repeated trials, but the learning was slower in lesion groups.

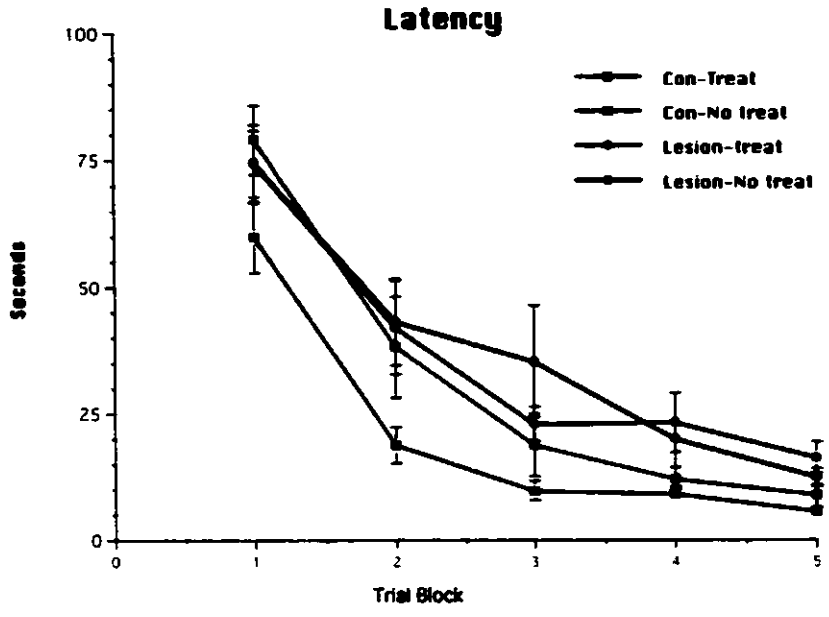


Figure 3. 4. Mean escape latency in the Morris water task for each of the five trial blocks.

A three-way ANOVA of mean total escape latency with lesion group, treatment, and sex as factors revealed an effect of group ($F(1, 18) = 10.66$, $p = .0043$), and of sex ($F(1, 18) = 4.88$, $p = .0404$), but not of treatment ($F(1,18) = 2.509$, $p = .1306$). Post hoc testing showed that there were sex differences in performance of spatial navigation, with lesion females being significantly worse than their male counterparts and the controls (Figure 3.6).

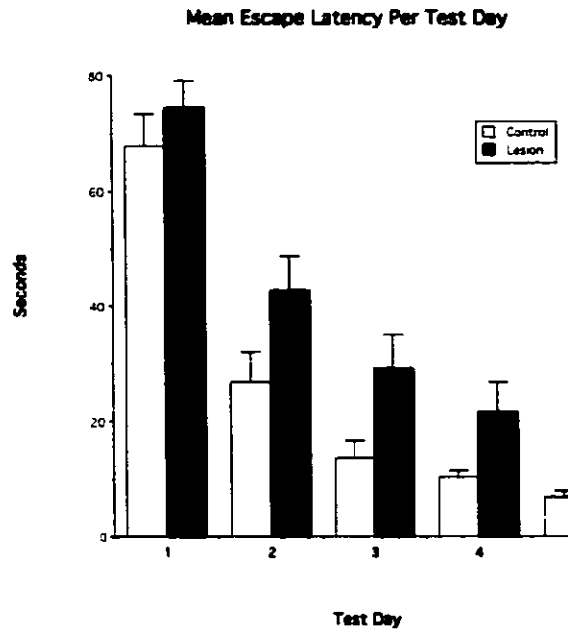


Figure 3. 5. Mean escape latency on the Morris water task per test day. Learning occurred in both control and lesion groups, but the learning was slower in lesion groups.

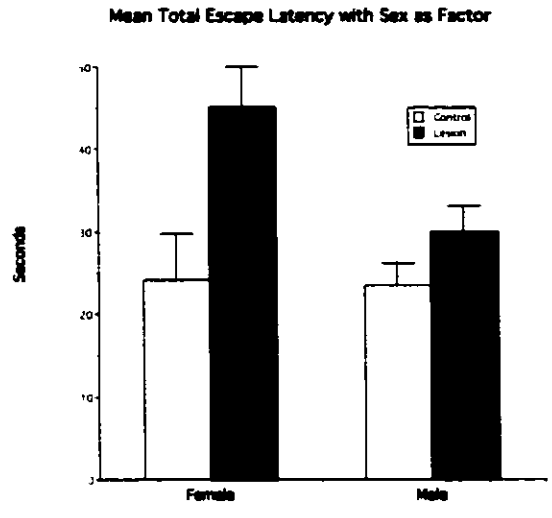


Figure 3. 6. Mean total escape latency on Morris water task with sex as a factor. Lesion females are significantly worse than their male counterparts, and of the controls.

In summary, tactile stimulation as a treatment to improve spatial navigation abilities was not beneficial. Lesion groups did improve with practice, but improvement was slower than with controls. Lesion females were significantly worse than other groups.

3. 3. 1. 2. Skilled Reaching

Bilateral motor cortex lesions had a significant effect in the ability to reach for food. The mean hit percent for each group was as follows: control/treated, 76%; control/non-treated, 59%; lesion/treated, 28%; and lesion/non-treated, 20%. A two-way ANOVA with lesion group and treatment as factors revealed a significant effect of group ($F(1, 22) = 60.74$, $p < .0001$), and treatment ($F(1,22) = 5.37$, $p = .03$), but no interaction ($F(1,22) = .758$, $p = .393$). Controls performed better than the lesion groups, and treated groups performed better than the corresponding non-treated ones (Figure 3.7). So there is improved reaching with tactile stimulation in both lesion and control groups.

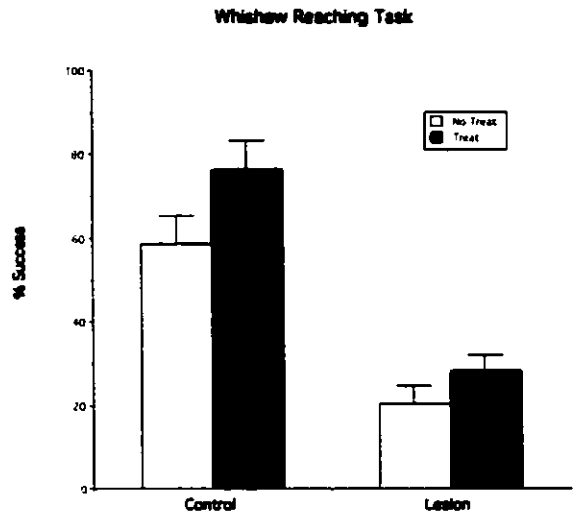


Figure 3. 7. Whishaw reaching task. Control groups performed better than the lesion groups and treated groups performed better than non-treated ones.

3. 3. 1. 3. Claw Cutting

When animals were perfused, measurements were taken of hindpaw nail length. A two-way ANOVA of mean nail length for both left and right hind paw revealed an effect of group ($F(1, 20)= 20.10$, $p= .00020$), an effect of treatment ($F(1, 20)=11.66$, $p= .0028$), and an interaction ($F(1, 20)= 4.48$, $p= .0471$). Treated groups were more efficient at claw cutting than non-treated ones, but only the treated controls were significantly better than the corresponding non-treated ones. Figure 3.8 shows the interaction of group x treatment. A follow-up three-way ANOVA examining sex as a factor, revealed an effect of sex x group ($F(1, 16)= 9.17$, $p= .0080$), and sex x treatment ($F(1.16)= 5.25$, $p= .0358$). Lesion females were more efficient at claw cutting than lesion males (Figure 3.9).

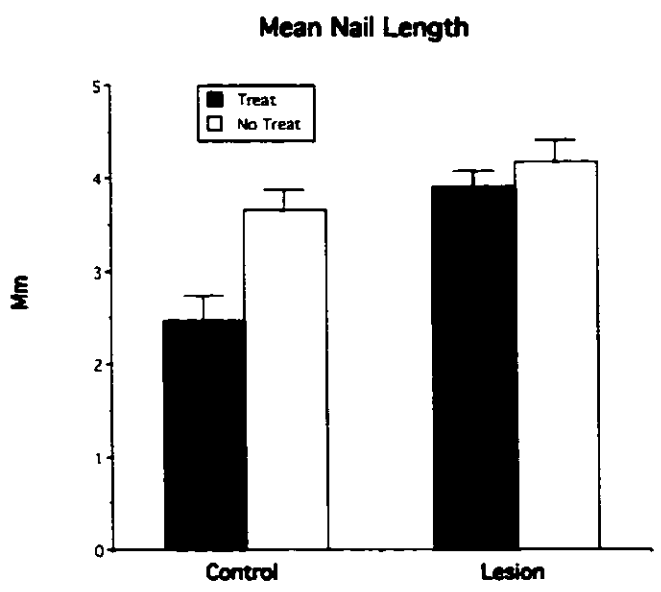


Figure 3. 8. Mean nail length comparing the interaction of group and treatment. Treated animals are more efficient at claw cutting.

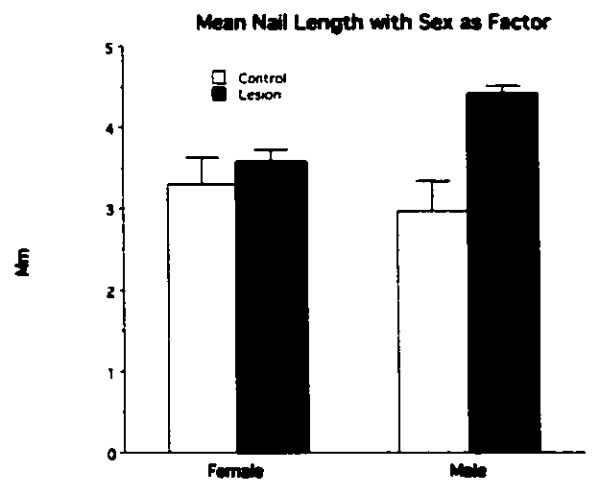


Figure 3.9. Mean nail length with sex as a factor. Lesion females are better at claw cutting than lesion males.

3. 3. 2. Anatomical Results

The lesions included the motor cortex bilaterally in all cases, with extension of the lesion into the anterior cingulate regions in some animals. A gross dorsal view of one control and two lesion animals is represented in figure 3.10, and figure 3.11 shows representative examples of a control and lesion animal in cresyl violet coronal sections from planes 1-3.

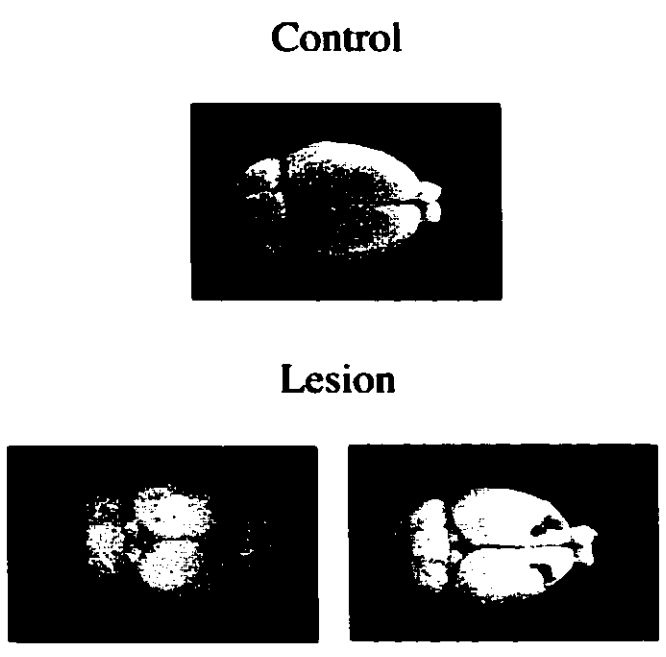


Figure 3. 10. Dorsal view of representative control and lesion animals.

In examining brain weights using a three-way ANOVA there was an effect of group ($F = (1, 17) = 10.55, p = .0047$), an effect of treatment ($F (1, 17) = 158.15, p < .0001$) but no interaction ($F (1,17) = .080, p = .78$). Fisher's PLSD revealed an effect of sex where brain weight was less in females than males. In both control and lesion groups, treated animals had larger brain weights than non-treated ones (Table 3.1).

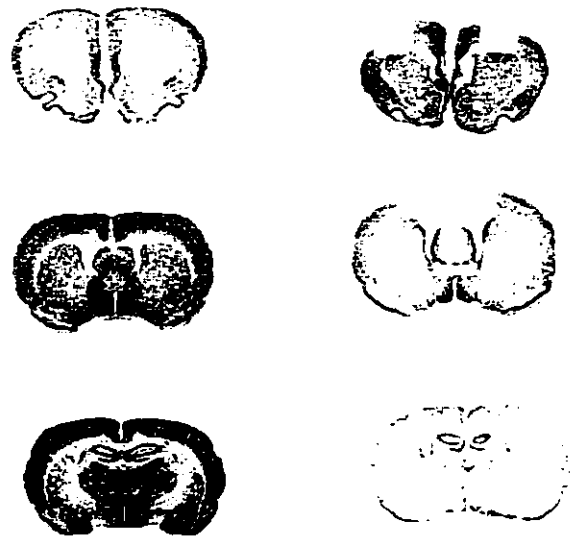


Figure 3.11. Cresyl violet coronal sections of representative control and lesion animals from planes 1-3. Differences in cortical thickness can be seen between the control and lesion animals.

Table 3. 1. Summary of brain weight. Treated groups weighed more than non-treated groups.

Group	Male	Female
Control No Treat	2.03 ± .01	1.97 ± .03
Control Treat	2.11 ± .003	2.01 ± .02
Lesion No Treat	1.71 ± .03	1.72 ± .03
Lesion Treat	1.80 ± .04	1.78 ± .04

Note: Numbers refer means ± SEM in grams.

Observation of cortical thickness in coronal views revealed damage corresponding to Zilles (1985) areas Fr1, Fr2, HL, FL, and portions of the corpus callosum. Figure 3. 12 shows coronal sections of a representative bilateral lesion animal. Statistical analysis examining the mean cortical thickness in planes 1-3 using a two-way ANOVA revealed an effect of group ($F(1, 21) = 24.22, p < .0001$), an effect of treatment ($F(1,21) = 4.05, p = .057$), but no interaction ($F(1,21) = 1.715, p = .205$). Fisher's PLSD showed significance between treated and non-treated groups.

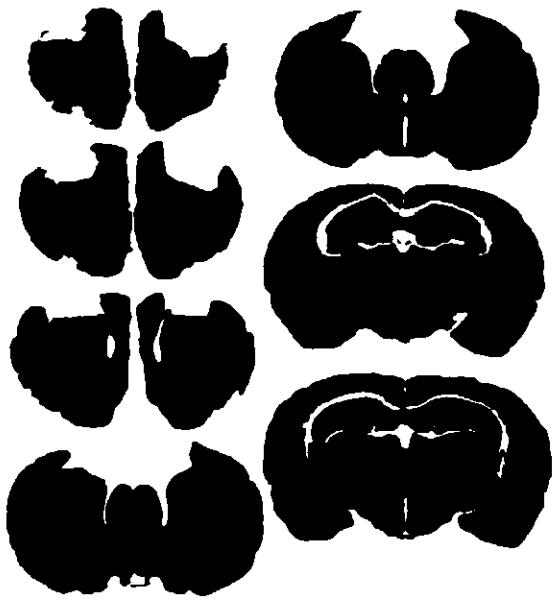


Figure 3. 12. Coronal sections of a representative bilateral lesion animal.

Interestingly, a one-way ANOVA looking at the effects of tactile stimulation on the lesion animals revealed that treated lesion animals had significantly thinner cortices from planes 1-3 than the non-treated lesion animals ($F(1, 11) = 6.85$, $p = .024$). The cortical thickness differences were seen primarily in plane 2 (Figure 3.13).

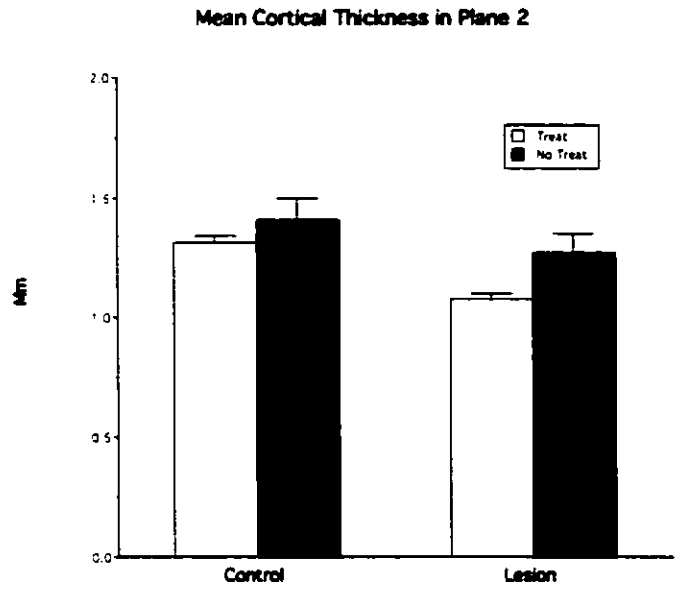


Figure 3. 13. Mean cortical thickness in plane 2 for control and lesion groups. Treated groups had smaller cortices than non-treated ones. Note that the treated lesion group had a significantly smaller cortical thickness than the non-treated group.

Further, there was a trend toward significance using a three-way ANOVA having lesion group, treatment, and sex as factors for plane 2. The trend occurred in the treatment x sex interaction ($F(1,17)=3.986$, $p= .062$) where treated male lesion animals had a smaller cortical thickness than females, yet

untreated male lesion animals had a larger cortical thickness than females (Table 3.2).

Table 3. 2. Summary of cortical thickness in plane 2 for males and females.

Group	Male	Female
Control No Treat	1.44 ± .14	1.37 ±.11
Control Treat	1.31 ± .02	1.33 ± .07
Lesion No Treat	1.43 ± .07	1.12 ± .05
Lesion Treat	1.04 ± .03	1.12 ± .02

Note: Numbers refer to means ± SEM of cortical thickness in mm.

3. 4. DISCUSSION

In this experiment, the response to tactile stimulation was examined as a treatment modality in bilateral motor cortex lesion P4 animals. The following abilities were tested in animals as adults: 1) spatial navigation while swimming in the water maze, considered a form of cognition; 2) reaching, a motor task; 3) claw cutting represented by hind paw nail length to examine biting and chewing abilities 4) brain weight differences; 5) cortical thickness differences and 6) sex differences.

There were three principal findings from this study: 1) Bilateral motor cortex lesions significantly affected latency in spatial navigation, reaching abilities, claw cutting skills, brain weights, and cortical thickness compared to controls; 2) Tactile stimulation as a functional treatment did not improve abilities in spatial navigation, but there were significant effects of treatment in skilled reaching, claw cutting, brain weights, and cortical thickness; and 3) Effects of sex occurred in spatial navigation, claw cutting, and cortical thickness measures. Each finding will be discussed in turn.

3. 4. 1. Bilateral motor cortex lesions, behavioral, and anatomic changes

In this study, results from testing on the Morris water maze and Whishaw reaching task were consistent with similar studies involving bilateral motor cortex lesions, as well as findings after damage to the medial frontal cortex (Kolb & Holmes, 1983; Kolb, 1995; Kolb et al., 2000a; Gibb, 2001). That is, animals with bilateral motor cortex lesions on P1-4 demonstrated severe motor deficits in adulthood as well as generalized deficits in cognitive functioning. However, studies where animals received these lesions in adulthood showed poor recovery on skilled motor behaviors but not on tests of cognitive function (Kolb & Holmes, 1983; Kolb et al., 2000a). In the current study, improvements in spatial learning occurred in lesion animals, but results were smaller and slower compared to controls. The other tests demonstrated reduced motor abilities, as well, consistent with results where a similar type of lesion was given (Whishaw et al., 1983; Kolb et al., 2000a; Gibb, 2001).

In the Kolb et al. study, widespread anomalous corticospinal projection fields into surrounding parietal cortex were found. These morphological changes corresponding with behavioral modifications to spatial learning, thought to be partially mediated by the parietal cortex, could reflect disruption of usual functions mediated by this cortex. If this outcome were the case, then the motor map located in the parietal cortex would be modified to reflect these corticospinal anomalies. It turns out that this modification occurs (see experiment 3), and may explain the reason why P4 animals have deficiencies in both motor and cognitive domains, or what Teuber (1975) refers to as "crowding."

3. 4. 2. Effects of tactile stimulation as a functional treatment

In skilled reaching, tactile stimulation improved the ability of both the control and lesion animals. For claw cutting abilities the same results occurred. Treated animals had significantly larger brain weights than non-treated ones. Finally, treated groups had thinner cortices than non-treated groups, and lesion treated animals had significantly thinner cortices than non-treated ones. These results were consistent with those found by Gibb (2001). The larger brain weight, coupled with the thinner cortices, then, may mean that the remaining cortical structures were denser morphologically, or that the subcortical structures were bigger.

Gibb (2001) has shown in studies on postnatal tactile stimulation that there is tremendous impact on recovery of function in perinatal lesion animals, particularly those having P4 medial frontal or posterior parietal lesions.

Morphologically, there was a decrease in dendritic length, but loss of arbor was reduced in the posterior parietal cortex lesion animals. Animals with perinatal lesions also showed a decline in spine density that was reversed by tactile stimulation, concurrent with increased acetylcholinesterase (AchE) levels. Secreted AchE serves to reinforce synaptic efficiency and promote growth and differentiation in postsynaptic surfaces (Rassmusson, 2000). This synaptic efficacy produces long-lasting increases in neural responsiveness and may therefore demonstrate lifelong enhanced potential for synaptic plasticity. By enhancing the sensory system through this environmental stimulation, then, life-long metabolic changes may influence subsequent motor abilities.

Schanberg and Field (1987) examined sensory deprivation stress and supplemental tactile stimulation in rat pups and pre-term neonates. Animal research suggested that lack of stimulation has deleterious biochemical and physiological effects in animals and that normal functioning can be reinstated through tactile stimulation. With tactile stimulation in the pre-term neonates there were improvements in weight gain, increased time spent awake, and increased motor behaviors when given a series of behavioral tests, suggesting biochemical and hormonal changes associated with this type of treatment.

3. 4. 3. Bilateral motor cortex lesions and sex differences

Effects of sex occurred in the spatial navigation task, where lesion females were significantly slower than all other groups. Females were more efficient at claw cutting abilities, however. As expected, because rats are sexually

dimorphic, the female brains weighed less than the male ones. Surprisingly, there was a treatment x sex interaction where untreated male cortical thickness measures were larger than untreated female measures, but thinner cortical thickness measures in treated lesion males compared to the female counterparts. Again, the effect of treatment may have altered either the density of cortical morphological structures or subcortical structures became larger.

4. EXPERIMENT 3: MOVEMENT REPRESENTATIONS FOLLOWING NEONATAL FRONTAL CORTEX DAMAGE

4. 1. INTRODUCTION

The rodent corticospinal system has been used to study the effects of damage in the developing central nervous system. Such studies have revealed a robust pattern of anatomical and physiological plasticity that appears to be enhanced in the developing brain. For example, motor impairments resulting from unilateral frontal cortex damage are less pronounced when produced perinatally than in adulthood (Whishaw & Kolb, 1988). The behavioral compensation is supported by the intact hemisphere (Barth & Stanfield, 1990) and involves an expansion of ipsilateral corticospinal projections (Castro, 1975; Huttenlocher & Raichelson, 1989). Although bilateral frontal cortex removal results in more profound motor deficits (Kolb & Cioe, 2000), these impairments are also not as severe as those observed in adult rats following similar damage (Napieralski, Banks, & Chesselet, 1998; Kolb, Cioe, & Whishaw, 2000a). The behavioral recovery observed following bilateral damage is likely supported by adjacent, intact tissue. Bilateral frontal cortex removal in neonatal rats also led to the appearance of corticospinal neurons within regions of parietal cortex that are normallly devoid of such cells in adulthood (Whishaw & Kolb, 1988; Kolb et al., 2000a). Furthermore, these animals exhibited deficits in several cognitive tasks that are believed to be due to a crowding (Teuber, 1975) of motor functions into neighboring, non-motor cortical areas.

The cognitive impairments and the displaced corticospinal neurons suggest a displacement of motor functions from frontal to parietal cortex. How this redistribution of function affects the organization of movement representations within the cortex is unclear. The present experiment, done in collaboration with Kleim, Ryder, VanderBerg, and Kolb (in press), used intracortical microstimulation techniques to examine the possibility that previously observed corticospinal neurons within parietal cortex represent functions typically found to be mediated by the motor cortex. Further, we examined how this functional reorganization correlated with motor performance in adulthood.

4. 2. METHODS AND PROCEDURES

4. 2. 1. Subjects and Surgical Procedures

Eleven postnatal day 4 (P4) male rats (Charles River/Long-Evans) from two litters were randomly assigned to either a lesion (n=6) or control (n=5) condition. All animals were anesthetized with procedures similar to experiment 2. Animals were group housed with same-sex littermates in stainless steel hanging cages on a 12:12 hour light/dark schedule and on ad lib food until behavioral testing on P60.

4. 2. 2. Skilled Reach Training

At approximately 60 days of age, all rats were food deprived to 90% body weight prior to training. Animals were pretrained in reaching cages similar to that described in experiment 2. A 4 cm wide and 5 cm deep tray filled with food pellets (45 mg; Bioserv) was mounted on the front of the cage. The rats were

required to reach outside the cage and retrieve pellets from the tray. All rats remained in pretraining until they had successfully retrieved 10 pellets (approximately 1 hour/day for 2 days). After pretraining, the rats were placed into a Plexiglas cage (11 cm X 40 cm X 40 cm) with a 1 cm slot located at the front of the cage. They were then trained to reach through the slot and retrieve a single food pellet located on a table outside the cage (Whishaw & Pellis, 1990). Training sessions lasted 15 minutes per day for 10 consecutive days. Each session was videotaped and later used to assess reaching performance. A successful reach was scored when the animal grasped the food pellet, brought it into the cage and to its mouth without dropping the pellet. The percentage of successful reaches [(# successful retrievals/the total # of reaches) X 100] was then calculated.

4. 2. 3. Electrophysiological Mapping

Within two days of the final training session, standard microelectrode stimulation techniques were used to derive topographical maps of movement representations within the motor cortex contralateral to the trained paw (Kleim et al., 1998). Briefly, animals were initially anesthetized with ketamine hydrochloride (70 mg/kg, i.p.) / xylazine (5 mg/kg, i.p.) and received supplementary doses of ketamine (20 mg/kg, i.m.) and acepromazine (.02 mg/kg, i.m.) as needed. A magnified image of the brain surface (230x) was captured using a video frame grabber. A 500 μm grid was then superimposed onto the image (Canvas 3.5) and viewed on a computer monitor in order to guide electrode placements. Microelectrode (600-800 kΩ) penetrations were made

using a hydraulic microdrive in 500 μm intervals at a depth of approximately 1550 μm (corresponding to cortical layer V). Stimulation consisted of a 40 ms train of thirteen, 200 μsec monophasic cathodal pulses delivered at 350 Hz from an electrically isolated, constant current stimulator. Pulse trains were delivered at a rate of 1 Hz. Evoked movements were examined while the animals were maintained in a prone position and the forelimb supported in a consistent position. At each site, stimulating current was gradually increased (up to 100 μA) until a movement could be detected (threshold current). If no movement could be detected at ≤100 μA, the site was defined as non-responsive. Movement of the elbow, shoulder, digit or wrist were all classified as forelimb movements and penetrations were made across the cortex until the entire forelimb area was surrounded by either non-response or non-forelimb movements (i.e., vibrissae, head, neck or jaw). The total area of the forelimb representation was then calculated using image analysis software (Canvas 3.5.4). Thresholds were also tallied to obtain a mean threshold current for forelimb movements. To examine the relative rostro-caudal position of the forelimb representation, the distance from each forelimb movement penetration site to Bregma was measured and averaged for each animal.

4. 3. RESULTS

4. 3. 1. Skilled Reaching

Lesion animals exhibited profound impairments on the skilled reaching task. Only four of the lesion animals could be trained to reach for food pellets.

The remaining two animals did not appear capable of producing reaching movements despite several additional training sessions and were not included in the behavioral analyses. All control animals, however, acquired the task and were trained for the entire 10 days. A Student's t-test (two-tailed, independent; $p < .05$) showed control animals to have a significantly greater percentage of successful reaches than lesion animals after 10 days of training ($t(8) = 2.87$; $p<.05$; Figure 4. 1).

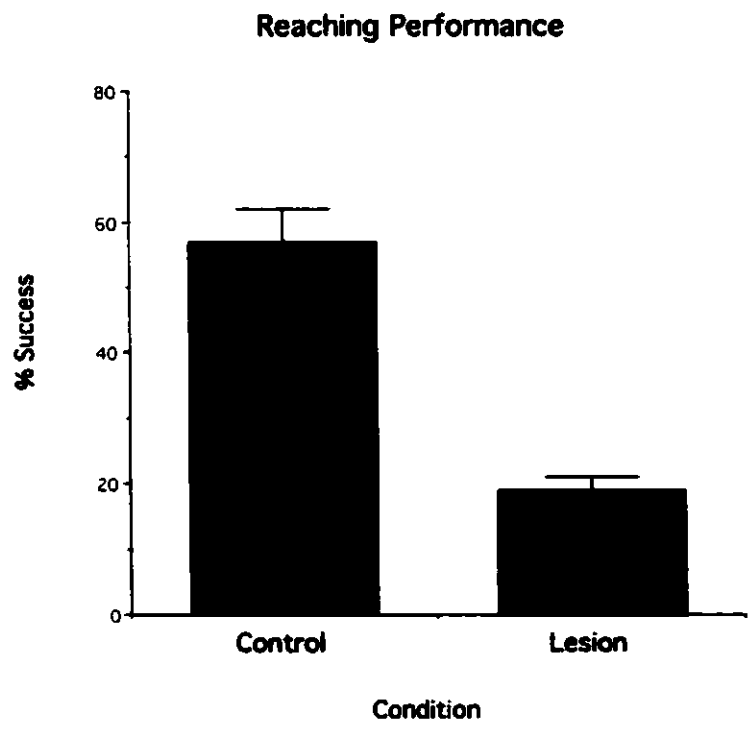


Figure 4. 1. Reaching performance on the skilled reaching task after 10 days of training.

4. 3. 2. Movement Representations

Forelimb representations in control animals were consistent with those previously reported from intact animals (Neafsey et al., 1986; Kleim, Barbay, & Nudo, 1998). The maps were characterized by a large caudal forelimb area separated from a smaller rostral forelimb area by jaw and neck representations. The caudal forelimb area was bordered medially by vibrissae, laterally by non-response sites and posteriorly by hindlimb respresentations (Figure 4.2A). In lesion animals, movement representations were highly disorganized and no distinction between rostral and caudal forelimb areas could be made (Figure 4. 2B,C).

The total cortical area occupied by forelimb representations was significantly smaller in lesion animals in comparison to controls ($t(9) = 4.67$, $p < .01$; Figure 4.3). Further, these representations were also significantly more posterior from Bregma ($t(9) = 2.69$, $p < .05$; Figure 4. 4) and had significantly higher mean threshold currents than controls ($t(9) = 6.84$, $p < .05$; Figure 4.5).

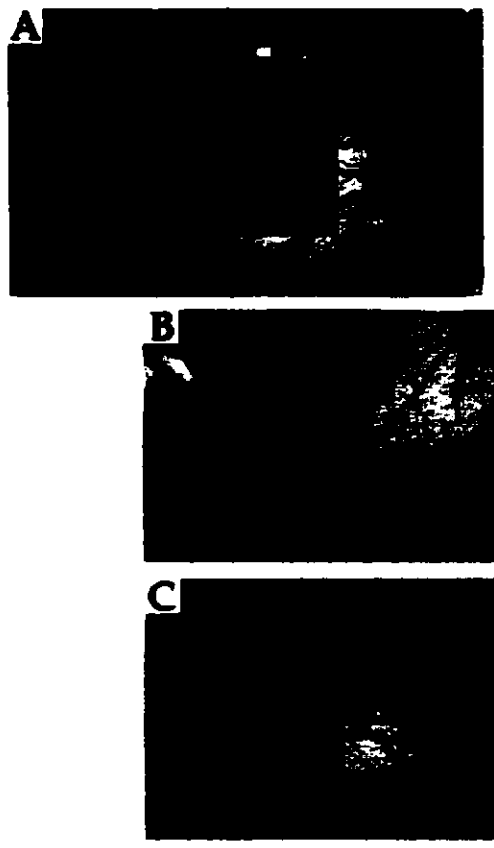


Figure 4. 2. Representative motor maps showing the organization of forelimb movement representations of a control (A) and two lesion animals (B, C). Forelimb movements are shown in green, vibrissae in purple, head/neck in yellow, hindlimb in blue and non-response sites are shown in gray. In comparison to controls, the maps of lesion animals appeared topographically disorganized, smaller and shifted into more lateral and posterior cortical regions (Bregma shown in red).

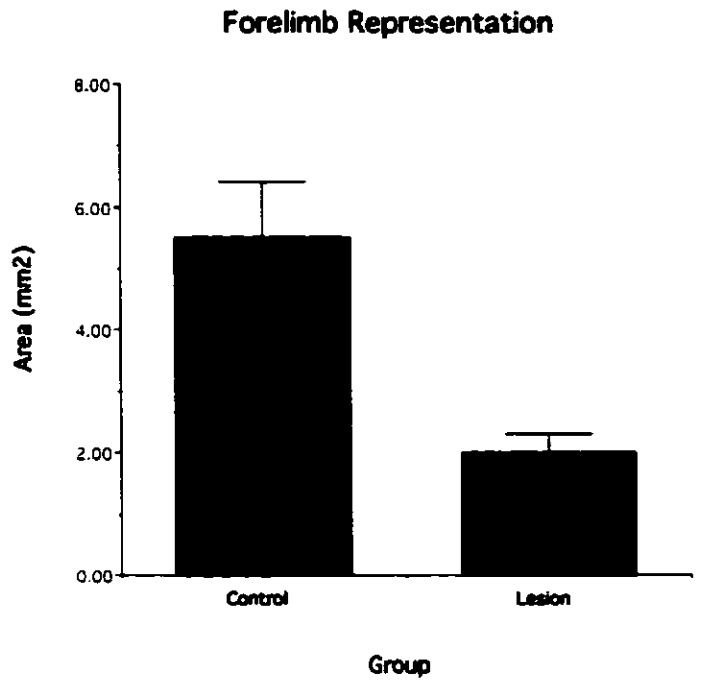


Figure 4. 3. Mean area of forelimb movement representations in control and lesion animals.

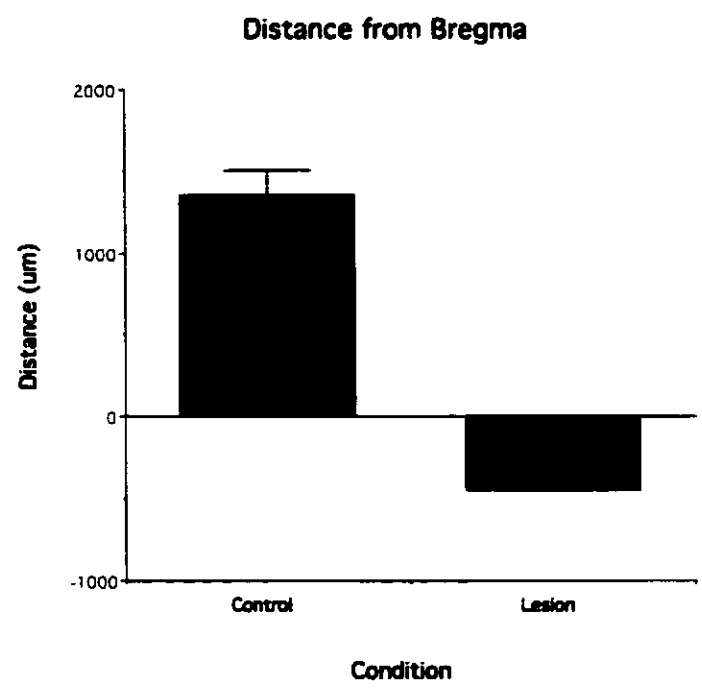


Figure 4. 4. Forelimb representations were significantly more posterior from Bregma.

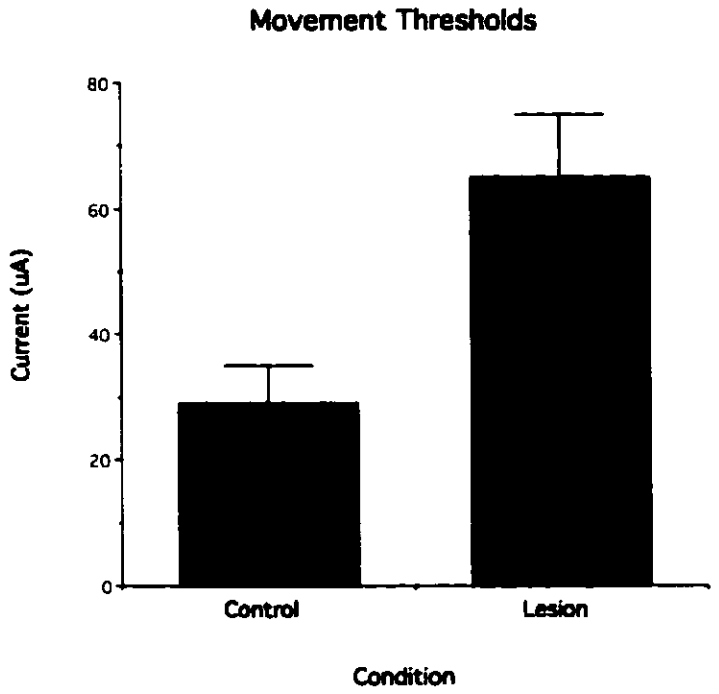


Figure 4. 5. Lesion animals had significantly higher mean threshold currents than controls.

4. 4. DISCUSSION

Functional compensation following brain damage is mediated, at least in part, by reorganization of residual neural tissue (Castro-Alamancos & Borrell, 1995; Nudo, Milliken, Jenkins, & Merzenich, 1996; Kolb, Cote, et al., 1997). The results of the present study demonstrate that removing lesions of neonatal cortex destined to perform motor functions in adulthood led to the appearance of movement representations within non-motor (parietal) cortex. The presence of movement representations within parietal cortex is consistent with previous work showing corticospinal neurons in this region following a similar manipulation (Whishaw & Kolb, 1988; Kolb et al., 2000a). However, these new motor

representations were abnormally small, topographically disorganized and required greater stimulation to elicit movement. The aberrant forelimb movement representations were also accompanied by profound impairments in skilled forelimb use.

Corticospinal neurons are found throughout the rat cortex during the first two weeks of development but are confined to frontal cortex by the third postnatal week (Bates & Killackey, 1984; Oudega, Varon, & Hagg, 1994). The presence of corticospinal neurons within the adult parietal cortex of P4 lesion animals likely reflects the maintenance of these exuberant connections rather than the generation of new neurons. The number of corticospinal neurons within the parietal cortex is also much lower than that seen within the motor cortex (Kolb et. al., 2000a) and may explain the increased stimulation required to elicit forelimb movements.

The results from this and other studies suggest that the degree to which functional compensation occurs following brain damage depends both upon the nature of the remaining neural tissue and the developmental stage at the time of insult. Compensation is more complete when it involves recruiting brain regions that already contribute to or are destined to perform functions similar to those of the lost tissue. For example, adult rats receiving total motor cortex lesions failed to show any significant motor recovery while those with subtotal lesions did show recovery (Whishaw & Kolb, 1988). This result suggests that residual motor cortex adjacent to the damage may functionally compensate for the lost tissue. Several experiments have shown reorganization of movement representations in

cortical tissue surrounding damaged regions that was associated with motor recovery (Glees & Cole, 1950; Castro-Alamancos & Borrell, 1995; Nudo, Milliken, et al., 1996). Further, infusion of nerve growth factor into the damaged hemisphere enhanced motor recovery, prevented dendritic atrophy and increased spine density in residual motor cortical areas (Kolb, et al, 1997). Recovery from subtotal motor cortex lesions was also abolished when a second lesion was produced in cortical areas surrounding the initial insult (Barth, Jones, & Schallert, 1990; Whishaw, 2000). Unilateral motor cortex lesions in the developing brain did not result in any significant motor impairments of the contralateral forelimb in adulthood (Barth & Stanfield, 1990). This compensation was accompanied by bilateral movement representations within the intact motor cortex (Kartje-Tillotson, Neafsey, & Castro, 1985; Kartje-Tillotson, O'Donoghue, Dauzvardis, & Castro, 1987). Thus, the motor cortex is better equipped to compensate for lost motor cortex than non-motor cortical areas even when in the contralateral hemisphere. When such tissue is not available, as in the case of bilateral damage, other brain areas are recruited. This compensation occurs at a cost as shifting motor functions into neighboring brain areas interferes with both motor function and the function of the recruited areas. Hence, animals with lesions similar to the P4 lesions in the present study showed deficits in cognitive tasks characteristic of adult animals with parietal cortex injury (Kolb et al., 2000a). Thus, although the developing brain is endowed with an increased capacity for compensation by shifting lost functions into residual neural tissue, this compensation can be both incomplete and maladaptive.

5. GENERAL DISCUSSION

5. 1. PLASTICITY AND RECOVERY OF FUNCTION

At no other time has so much been known about the mechanisms underlying neuronal death and brain injury. With this knowledge, one of the challenges, both for behavioral neuroscience and rehabilitation medicine is first to agree on an operational definition for "recovery of function," be it complete restitution, partial restitution, or compensation. The second goal, then, would be to find ways to stimulate the brain to compensate for injury to the cerebral cortex and improve behavioral function. It is now clear that the brain is capable of considerable plasticity and that cerebral organization and resulting behavioral change may be at least somewhat flexible.

From the plethora of studies on the subject of plasticity, there are four main conditions where this phenomenon occurs: developmental plasticity, activity-dependent plasticity, plasticity of learning and memory, and injury-induced plasticity (John F. Kennedy Center for Research on Human Development, Vanderbilt University, 2000). The series of experiments in this thesis demonstrated plastic changes in all four conditions, corresponding with postlesion functional improvements.

There are two main questions in research in plasticity and recovery of function. First, what are the neural mechanisms underlying the observed compensatory changes? Second, is it possible to enhance these changes and thus potentiate recovery? In order to add to the converging evidence in response

to these questions, the following experiments were done: 1) a comparison of skilled reaching (structured training) versus an enriched environment (functional training) as treatment modalities with and without administration of FGF-2 in unilateral motor cortex lesion adults; 2) tactile stimulation as a treatment modality for recovery of motor and cognitive functions in P4 bilateral motor cortex lesion rat pups; and 3) cortical mapping of the motor representation in bilateral motor cortex lesion adult rats after being trained in skilled reaching. Many of the proposed neural mechanisms underlying the four types of plasticity mentioned can be used to explain the results of these experiments.

5. 2. ENVIRONMENTAL ENRICHMENT AND RECOVERY OF FUNCTION

Many studies have shown the behavioral, anatomical and morphological effects of EE both in normal and lesion animals (Hebb, 1949; Bennett et al., 1964; Bennett, 1966; Ohlsson & Johansson, 1995; Johansson, 1996; Whishaw, 2000; Witt-Lajeunesse & Kolb, in press). These enhanced effects include increases in brain weight, cortical thickness, neuron size, dendritic branching, number of dendritic spines per unit length of dendrite, glial proliferation, protein content, cortical cholinesterase activity, angiogensis, and production of neurotrophic factors. Behavioral changes include improvements in motor abilities, cognitive functioning, and overall exploratory activity. For this reason, the EE environment was considered a functional treatment because of the overall enhanced stimulation provided in this condition. As an example, Ohlsson and Johansson gave middle cerebral artery occlusive strokes to hypertensive rats

and placed them in EE housing either before or after surgery. Significantly better performances were noted on motor testing compared to rats living in standard cage housing. Even if there is a delay in transfer to EE housing, improvement occurs in motor performance. In addition, Johansson and Ohlsson (1996) found that a comparison between an EE, social interaction and physical activity in the form of wheel running, revealed that an EE combined with social interaction resulted in the best performance.

Results of experiment 1, indeed, support other studies involving EE (functional training), particularly in the Whishaw reaching task and the Schallert spontaneous vertical exploration task. It is interesting to note, however, on the other subtests given that EE animals were not significantly better compared to the other training groups, except when FGF-2 was included with the EE. This study also supports one by Grabowski, Brundin, and Johansson (1993) indicating that the increase in motor activity in the EE housing alone may benefit more generalized forepaw use. In experiment 1,The EE housing, however, did not aid in the use of fine motor movements of the wrist and digits as detected by the single pellet reaching task. In summary, EE alone aided functional improvement in some generalized motor movements, including a decrease in non-dominant paw use and increased abilities in skilled reaching.

5. 3. SKILLED REACHING AND RECOVERY OF FUNCTION

Skilled reaching as a treatment for recovery of function has been used in both primate and rodent studies (Nudo, Milliken, et al., 1996; Biernaskie &

Corbett, 2001; Goertzen, VandenBerg, Yamagishi, Neufeld, & Kleim, 2001). Changes in the motor map have been seen in both the lesion and intact hemispheres, implicating underlying mechanisms that include enhancement of corticocortical connections from frontal to parietal cortex, as well as increases in dendritic spine, and synaptic complexity in corticospinal connections. The training protocols vary (i. e., skilled reaching for pellets, skilled reaching as an exercise, and an acrobatic obstacle course). Overall, it appears that there are angiogenic improvements in some activities involving exercise-induced recovery. Other studies demonstrate dendritic enhancements as well as changes in the cortical map with subsequent functional improvements.

When there were aspiration lesions as large as those performed in experiment 1, specific skilled reaching did not significantly improve unless FGF-2 was administered postlesion, despite the fact that there remained intact corticospinal connections. Having the training for only one hour per day, and in many cases only 30-45 minutes, due to gustatory satiation by the animals, was not enough to demonstrate significant compensatory improvement. If we look at skilled reaching qualitatively in the single pellet reaching task, we see that actual compensatory movements were occurring (i.e.: increased tongue use to retrieve pellets, decreased sensory awareness in the affected limb, poor grasping, and poor supination abilities). Biernaskie and Corbett (2001), however, have combined skilled reaching with an EE housing condition and have found morphological changes corresponding to increased rehabilitation-induced recovery of function. In this case, skilled training occurred more than one hour

per day. In addition, Kleim et al. (1996), comparing a learned acrobatic condition to mere motor activity supported the notion that motor learning leads to increases in synapse number in the motor cortex demonstrated by changes in biochemical processes and correlating with behavioral improvement, more so than the motor activity alone.

In the case of the bilateral motor cortex lesion animals in experiments 2 and 3, there was little possibility of the connections in the intact hemisphere helping to compensate for the injury, because of the nature of the lesion (Kolb et al., 2000a). Experiment 3 showed that the motor map had moved into the lateral and posterior parietal area in these animals. This alteration of the motor map may confirm what Teuber (1975) speaks of as "crowding" because the skilled reaching performance did get better, but at the expense of other cognitive functions (i.e., spatial navigation). The results from experiment 1, then, indicate that ST alone in general is not significantly beneficial for rehabilitation-induced recovery of function, and that in experiment 2, skilled reaching improved at the expense of other cognitive functions.

5. 4. FGF-2 AND RECOVERY OF FUNCTION

In the intact adult rat, FGF-2 is present in neurons, glia, the vascular basement membrane of blood vessels and in the ependymal cells lining the ventricles (Cuevas, Gimenez-Gallego, Martinez-Murillo, & Carceller, 1991). Other experiments involving injury-induced plasticity revealed changes resulting in an increase in endogenous FGF-2 (Rowntree, 1995; Rowntree & Kolb, 1997)

in specific targeted areas and within a specified time frame postlesion. Finklestein's group (CNS Growth Factor Research Laboratory, Harvard University, 2001) discovered that there is a biphasic pattern of FGF-2 gene expression in ischemic stroke. The first peak occurring before neuronal death may be a protective response of surviving neurons. The second peak, well after neuronal death, may produce a protective response involving reactive astroglia with subsequent cellular and synaptic organization. As well, Gomez-Pinilla, So, and Kesslak (1998), have found that FGF-2 may support cellular plasticity associated with learning and memory, going beyond that of just injury repair.

Studies involving exogenous administration of FGF-2 show that it reduces infarct size and supports functional recovery if given within hours of the brain insult, and improved functional behavior if given at later time points (Kawamata, Speliotes, & Finklestein, 1997). FGF-2 has been found to be a potent vasodilator, suggesting that regional cerebral blood flow may subsequently be altered, thereby reducing infarct size.

In experiment 1, the combination of FGF-2 and the EE housing condition improved motor activity, vertical exploration, forepaw inhibition during swimming, tongue extension, claw cutting abilities, and brain weight. There are several possible mechanisms occurring here. Because there was an increase in the cortical thickness in the intact hemisphere of the animals, there could have been an increase in endogenous production of FGF-2 due to the EE experience. Because this EE experience is perceptually and socially stimulating, motorically demanding, and is continuous for several months, that varied experience may

have enhanced endogenous FGF-2, which then enhanced cortical thickness, consistent with results by Gomez-Pinilla et al. (1998). As well, damaged cells were bathed in exogenous FGF-2 for 7 days, a time period post-injury where neurite promoting factors, glia proliferation, and reactive synaptogenesis is occurring (Nieto-Sampedro & Cotman, 1985). An important finding in this study was the fact that FGF-2 alone did not improve functional abilities, except in forepaw inhibition during swimming. In fact, in some cases behavior was worse, or animals reverted to the use of the undamaged limb and were unable to use the affected limb, possibly an indication of "learned non-use." However, a parallel study was done using animals that were given unilateral motor cortex lesions, received no training or handling for four months, then were trained on skilled reaching and received FGF-2. This study also revealed a trend in improving forepaw inhibition during swimming. Further testing is needed, however, to tease out whether the effects of time or the delayed effects of FGF-2 were beneficial in this group.

The results from experiment 1 have important implications in treatment for brain injury in humans, particularly with the increase in lab and clinical studies involving pharmacological interventions. It would appear that the combination of pharmacological intervention with rehabilitation therapies such as physical, occupational and speech-language therapy would be most beneficial for behavioral improvement. Based on experiment 1 then, it is the combination of EE (active functional rehabilitation) and FGF-2 that improved overall behavior. It would be interesting to study the effects of the combination of FGF-2, EE

housing, and skilled reaching in animals to determine whether further behavioral recovery can occur.

5. 5. TACTILE STIMULATION AND RECOVERY OF FUNCTION

Gibb (2001) has shown in an elegant set of experiments the positive effects of tactile stimulation in prenatal and postnatal lesion rat pups with subsequent improvements in behavioral function as adults. Overall, results indicated that there was high behavioral recovery and increased acetylcholinesterase levels in P3 frontal lesion animals when the pregnant dam was given tactile stimulation (prenatal stroking). In P4 frontal and parietal lesion animals given postnatal tactile stimulation, results revealed high behavioral recovery corresponding with high dendritic and spine growth. In these and other studies in the Kolb lab, more treatment-induced improvement occurs in the animals that are most severe (P4) as opposed to the P7-10 groups, where treatment-induced functional recovery is less. This greater improvement may be due to the fact that the cellular events occurring at P10 include synaptogenesis, promoting spontaneous recovery, and that less additional external environmental stimulation is therefore beneficial.

Tactile stimulation proved to be effective in improving both lesion and controls in skilled reaching, but not in spatial navigation. The lesion animals continued to have increased difficulties with the task. Experiment 3, however, indicated that the cortical map moved more posteriorly and laterally into the parietal lobes with non-treated animals. Tactile stimulation, then, appears to be

more helpful for task-specific activities such as skilled reaching supported more by corticospinal connections, as opposed to spatial navigation supported more by corticocortical connections. The map had changed at the expense of normal functions remaining in the parietal cortex. It remains to be seen how the cortical map would be altered with the added treatment.

Experience in combination with gonadal hormones produces different effects in the behavior and brain morphology of males and females (Juraska, 1990). Delineating differences becomes more complex when effects of lesion are added. In experiment 2, the significant effects of tactile stimulation treatment and sex occurred in brain weights, where treated males and females had larger brain weights than untreated counterparts. In contrast, there was a significant difference between untreated and treated lesion males in cortical thickness measurements where treated lesion males had smaller cortical thickness measures.

It is interesting to note the discrepancy in brain weight and cortical thickness between the tactile stimulated animals. Treated animals had significantly larger brain weights than non-treated animals, yet had decreased cortical thickness, implying either an increased density in cortical morphology or larger subcortical structures such as the thalamus. These results were consistent with studies done by Gibb (2001).

Several possible mechanisms may account for the tactile stimulation treatment effect. Gibb (2001) found that there was an upregulation of acetylcholinesterase (AchE) with this treatment paradigm. AchE has been

implicated in alterations of synaptic efficacy, growth and differentiation of neurons, and mediating cortical plasticity (Rasmusson, 2000). This synaptic efficacy produces long-lasting increases in neural responsiveness and may therefore demonstrate lifelong enhanced potential for synaptic plasticity. By enhancing the sensory system through this environmental stimulation, then, life-long metabolic changes may influence subsequent motor abilities. Another possible mechanism may be the injury-induced increase in FGF-2 (Rowntree & Kolb, 1997). Because FGF-2 is found in other organ systems as well as the nervous system (Kawamata, Speliotes, & Finklestein, 1997), it could be the case that FGF-2 expression is increased in the skin due to the treatment. In sum, the infant-damaged brain is especially responsive to tactile stimulation that is initiated in infancy.

5. 6. HUMAN STUDIES ON RECOVERY OF FUNCTION

The focus of this thesis has been mainly on animal studies involving cortical reorganization, injury-induced plasticity, activity dependent plasticity, and pharmacological interventions to increase functional improvements. Several questions arise as to the transfer to clinical studies on recovery of function. First, are there studies to indicate pharmacological advances in recovery of function from brain injury, specifically with FGF-2? Second, are there studies that look at cortical reorganization combined with activity-dependent recovery of function? Third, is there a human equivalent to an enriched environment? Several studies will be discussed briefly that touch on these questions.

Clinical studies involving pharmacological treatments such as FGF-2 were mentioned in the introduction. In a North American phase II/III clinical trial, intravenous FGF-2, or its trade name trafermin, targeted 302 patients with a thromboembolic stroke (Internet Stroke Center, Stroke Trials Directory, 2001). Admission criteria included onset of stroke within 6 hours of drug administration and a minimal score of 4 on the National Institute of Health (NIH) stroke scale. The North American study was terminated in October, 1999 because of an unfavorable risk to benefit ratio when comparing different dosages to the placebo. Mortality at 90 days was 25-29% using the drug, versus 13% using the placebo. The European phase of the study is continuing however.

With regard to studies in activity-dependent recovery of function, Taub and colleagues have spent years focussing on treatment in the form of constraining a good limb after deafferentation in primates, then later in humans. Taub et al. (1993) believed that in lesion animals, the movements of the intact limb inhibited movement of the affected limb, and that animals learned not to use the affected limb after several failed attempts ("learned non-use"). The treatment was called constraint-induced therapy (CIT). In his work with humans, he chose a select group of patients who passed strict admission criteria including: 1) at least 1 year poststroke; 2) less than 75 years old; 3) no significant balance problems during ambulation; 4) no excessive spasticity; 5) no significant secondary medical conditions; 6) no detrimental cognitive deficits; and 7) highly motivated. In these patients, the good limb was constrained 90% of waking hours for 12 consecutive days. A control group was used as well, having 12

days of rehabilitation consisting of passive range of the affected arm, and attention focussed visualization. Results indicated significant improvements in tests of motor tasks and actual daily arm use, even 2 years later. The control group regressed to pre-study levels within 1 year. Further testing (Taub et al., 1999) revealed that a family of different motor therapies could overcome learned disuse after stroke, but minimum motor skill, adequate cognitive levels, and increased motivation by patient/family are required. Taub reported that it was the shaping or grading of the selected activities that improved the restoring of movements. He has also used CIT with lower limbs of patients having a stroke, incomplete spinal cord injury, and fractured hips. Recently, a study has shown a constraint-induced form of treatment in a chronic aphasia group as well (Pulvermuller et al., 2001). To investigate the neuroanatomical changes produced by CIT, a study by Liepert, Bauder, Miltner, Taub, & Weiller (2000) was done with 13 stroke patients having chronic stroke symptoms. Researchers used focal transcranial magnetic stimulation (TMS) to map the cortical motor output area of a hand muscle bilaterally, prior to and after the treatment. TMS involves the use of a focussed magnetic field to non-invasively map the cortical representation areas of muscles in motor areas on the scalp. Results indicated that the cortical representation area of the affected hemisphere became larger after treatment, suggesting a recruitment of adjacent brain areas. This change corresponded with increased functional improvement and remained in follow-up evaluation 6 months later. There was also a shift in the homotopic area of the intact hemisphere, where the cortical area sizes became almost identical

bilaterally. It appears, then, that CIT can be a useful treatment in a select group of patients. As well, it demonstrates actual neurological changes corresponding to functional recovery.

Is there a human equivalent to an EE? As in animal studies, this type of setting in humans should include one in which there is an increase in social interaction, functional treatment on a daily basis, multiple senses being accessed (i.e., sound, smell, vision, touch) and a variety of motor and cognitive activities. A study by Jorgensen et al. (2000) has shown that there can be a medical model that may mimic an EE. This study involved looking at the effects of treatment and rehabilitation of patients with acute stroke in a dedicated stroke unit, which can be considered a form of EE. There were 1241 consecutive stroke patients from two separate communities in Copenhagen. Completely unselected stroke patients of one community went to a general ward/medical unit, while the patients from the second community went to a dedicated stroke unit. Outcome measures included initial, 1 year, and 5 year mortality rates, poor outcome (death or discharge to a nursing home), and length of hospital stay.

On the dedicated stroke unit a plan for evaluation, medical treatment, and rehabilitation was made on admission. Standard diagnostic evaluations included a routine blood test, ECG, chest radiography and a CT scan. Aspirin was given to all ischemic stroke patients, and anticoagulation was given if appropriate to patients having atrial fibrillation. In constrast, patients on the medical wards were given traditional treatments offered to Danish hospitals without a stroke unit. There was no standardized program for evaluation except a CT scan and aspirin

to all ischemic stroke patients. Physical, occupational, and speech-language therapy were given when prescribed by the physician. Results revealed that the relative risk of initial death, poor outcome, 1 year, and 5 year mortality rates were reduced by 40% in patients treated on the stroke unit compared with the general ward. Those who benefited most were patients with the most severe strokes, and those who benefited least were patients with mild or moderate strokes. Interestingly, this observation corresponds with results from several animal studies that Kolb, Brown, Witt-Lajeunesse, and Gibb (2001) have done with treatment-induced recovery of function. Length of hospital stay was reduced 2-3 weeks, except for those with the most severe strokes. In this case, it is important to realize that marked improvement in outcome shown in this study comes from the team approach, and the synergistic efforts of medical, nursing, rehabilitation, patient and family participation. This would be the most favorable type of setting to further assess patients having pharmacological interventions such as FGF-2, and to use TMS or neuroimaging techniques to differentiate activity-dependent or injury-induced plasticity correlated with functional recovery.

5. 9. SUMMARY AND WHERE DO WE GO NEXT?

A summary of results from experiments 1-3 is shown in figures 5.1-5.3. Several additional experiments based on the results from this thesis are either in process or could be proposed. A morphological analysis of the brains in experiment 1 are in process to determine neuronal changes resulting from various treatments. In addition, another study is underway to look at the effects

of exposure to an enriched environment for only 2-3 hours per day. Rehabilitation-induced recovery could be examined using the combination of FGF-2, skilled reaching, and the EE housing. While FGF-2 has been attempted with P3 bilateral medial frontal lesion animals as a treatment with favorable results (Kolb, Witt-Lajeunesse, Schlachter, and Gibb, 2000), this treatment paradigm could also be done with animals having bilateral motor cortex lesions, both with and without tactile stimulation as an additional treatment. Finally, from a clinical perspective, pharmacological interventions and/or constraint-induced therapy on a dedicated stroke unit might provide valuable treatment and insights into using among the best internal and external environmental conditions to date.

Information from these and other studies on rehabilitation-induced recovery of function will enhance our knowledge to improve treatments in individuals suffering from brain injury, and increase knowledge of the mechanisms underlying recovery. To this end, we need to continue to focus on these questions

1. How can the external environment be manipulated to maximize functional recovery?
2. How can the internal environment be turned on to provide maximal recovery of function?
3. What single therapy or combination of therapies would work to maximize current function and minimize further damage, thereby increasing overall recovery of function?

By examining then, the mechanisms underlying each of the above, we will

begin to utilize the best treatment protocols for individuals, and to know the therapeutic windows for optimal non-pharmacological and pharmacological interventions after brain injury.

Treatment Condition		Lesion/Group Condition	Significant Improvement
[redacted] (ST)	+	Lesion	-No Effect
[redacted] (ST)	+	Lesion + FGF-2	-Skilled Reaching -Single Pellet
[redacted] (FT)	+	Lesion	-Skilled Reaching -Spontaneous Vertical Exploration
[redacted] (FT)	+	Lesion + FGF-2	-Skilled Reaching -Spontaneous Vertical Exploration -Forepaw Inhibition During Swimming -Tongue Extension -Single Pellet -Claw Cutting -Brain Weight -Cortical Thickness (Intact Hemisphere)
No Treatment	+	Lesion + FGF-2	-No Effect

Figure 5. 1. Summary of results from experiment 1.

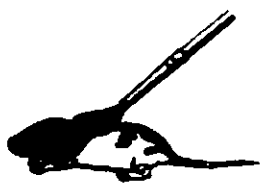
Treatment Condition		Lesion/Group Condition	Significant Effects
	+	Control/ Lesion	↑ Skilled Reaching ↑ Claw Cutting ↑ Brain Weight ↓ Cortical Thickness

Figure 5. 2. Summary of results from experiment 2.

Intracortical Microstimulation		Condition	Significant Effects
[redacted]	+	Lesion (Untreated)	↓ Skilled Reaching ↓ Total Cortical Area Representing Forelimb Area ↑ Forelimb Area More Posterior from Bregma ↑ Mean movement threshold currents

Figure 5. 3. Summary of results from experiment 3.

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