FACTORS INFLUENCING FUNCTIONAL RECOVERY FOLLOWING HEMIDECORTICATION IN RATS

MORGAN M. DAY
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THESIS ABSTRACT

Large neocortical lesions, such as hemidecortication, are detrimental for motor and cognitive skills. This thesis investigates the effect of age at the time of lesion on functional outcome. Attempts were then made to improve the outcome by using two simple treatments, tactile stimulation and Fibroblast Growth Factor-2 (FGF-2). The functional outcome of animals was measured using a series of behavioural tests (Morris water task, skilled reaching, forelimb placing during spontaneous vertical exploration, and the sunflower seed task). A qualitative difference was noted between animals that received hemidecortication at postnatal day ten (P 10) versus animals that received a hemidecortication in adulthood (postnatal day 90, P 90). When the tactile stimulation treatment was used on animals that received P 10 hemidecortication, cognitive and motor improvements were noted. The same was not true for injections of FGF-2. When given after P 10 hemidecortication, this treatment impaired the cognitive abilities of rats in the Morris water task. There are two main points from this project: 1) overall functional recovery is not better or worse but simply different based on the age at which the trauma occurred and 2) treatments have varied success with different types of brain injury.

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DEDICATION

For Brooke,

Thanks for reminding me that research really does change lives.

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ABBREVIATIONS

ANOVA	Analysis of Variance
C	Centigrade
cm	Centimetres
Con	Control
E	Embryonic
FGF-2	Fibroblast Growth Factor-2
g	Grams
Hemi	Hemidecorticate
hr	Hours
ICMS	Intracortical Microstimulation
kg	Kilogram
m	Meters
ul	Microlitres
mg	Milligrams
ml	Milliletres
mm	Millimetres
NT	No Treatment
P	Postnatal
TS	Tactile Stimulation

1. GENERAL INTRODUCTION

Brain injury is one of the leading causes of long-term disability, reduced quality of life, and reduced socioeconomic status in Canada. Brain injury can occur at any time throughout our life span but behavioural consequences are in part dependant on the age of the person incurring the injury. For example, damage to the left hemisphere in adulthood may cause severe language disturbance, but the same injury in infants rarely leads to permanent language deficits (Rasmussen & Milner, 1977). Similarly in laboratory rats, brain injury in the second week of life appears to have a less detrimental effect on behaviour during adulthood than comparable injury later in life. Exploring the factors that allow better functional recovery following early brain injury may identify novel treatments and therapies for brain insult both later in life as well as during development. The goal of this thesis is to (1) investigate the effects of large neocortical lesions in rats during development on functional outcome in adulthood and (2) explore the benefits of treatments that have previously been reported to be effective for the treatment of focal lesions in laboratory animals.

Brain Development

The stages of brain development are described for both humans and rats to provide a reference point between the two groups. All mammals go through the stages of neuronal development in a similar order. It is the age at which each stage occurs that differs between species.

Human Brain Development.

The development of the brain has many steps. Three weeks after conception, a flat sheet of cells has formed. The ends of the sheet eventually roll up

to form the neural tube. At about 100 days, the nervous system becomes recognizably human. Sulci and gyri do not develop until about seven months after conception. At birth the brain resembles that of a human adult but has a slightly different cellular structure.

Neurons develop in a precise sequence of steps (Table 1-1). Cell birth or neurogenesis begins at about seven weeks after conception and is largely complete after twenty weeks of development. Cell migration starts immediately after the first cells are produced but continues for six weeks after neurogenesis is complete. Cells migrate along "pathways" called radial glial cells. The brain develops starting with the inner layers and ending with the outer layers and cells in the cortex form six distinct layers.

The migrating cells or neuroblast are yet to differentiate. Once these cells reach their proper location, they differentiate into neurons. Cell differentiation is essentially finished by birth, but cell maturation, which includes the growth of dendrites, axons, and synapses lasts for years.

To form a neural-network, neurons must create connections with cells around them. During the neuronal maturation stage, neurons extend their dendrites and axons away from the cell soma to reach appropriate targets. Each dendritic branch then begins to form spines, which will be the point other neurons will communicate to or "synapse".

Similarly, an axon must follow various molecules, such as trophic factors, that attract or repel it to find the proper target on which it can synapse.

One of the most surprising events that occur during human brain development is cell death. Because the brain overproduces cells in the brain, programmed cell death or

"apoptosis" commences at the end of a child's first year of life to remove incorrect or unnecessary cells and synapses.

The final stage of brain development is myelogenesis. This process could last up to 18 years of age. Myelin sheaths insulate neuronal axons with a rationale similar to the plastic casing on an electrical wire.

Table 1-1. The Stages of Brain Development.
(Kolb & Whishaw, 2003)

1) Cell birth (neurogenesis)
2) Cell migration
3) Cell differentiation
4) Cell maturation (dendrite and axon growth)
5) Synaptogenesis (formation of synapses)
6) Cell death and synaptic pruning
7) Myelogenesis

Rat Brain Development. Rats are born after 22-23 days gestation. The neuronal proliferation of a rat continues until birth and cell migration continues for five to seven days afterwards.

Because the rat undergoes its final stages of development postnatally, it makes an ideal model for studying prenatal brain damage. In other animal models, such as sheep, prenatal damage must be performed while the animal is still in *utero* because brain development is largely complete when the animals are born. In rats a model of prenatal damage can be done postnatally. This is an easier process for the surgeon. On the negative side, the post-lesion environment is very different, as the rats are no longer exposed to the womb.

Comparisons of the two systems (Figure 1-1). It is difficult to compare rat and human development because of the fissurization of the human cortex. In its latest stages of

foetal development, the human brain undergoes a large expansion, which is characterized by numerous fissures and gyri. The rat brain however is lissencephalic, meaning it has a smooth cortex (Bayer, Altman, Russo, & Zhang, 1993). Thus, visually, the two brains are very different when fully mature.

Humans and rats also differ between their periods of intense dendritic growth. For humans this period begins in the early postnatal months and can last up to 18 months of age whereas rats have show the same growth from postnatal day 10 to 20.

The rat and human brains also have developmental similarities. The nervous systems of both species go through the same embryonic stages; a neural plate followed by a neural groove and finally a neural tube. Both groups go through the same stages of brain development (Table 1-1) but the length of each stage differs.

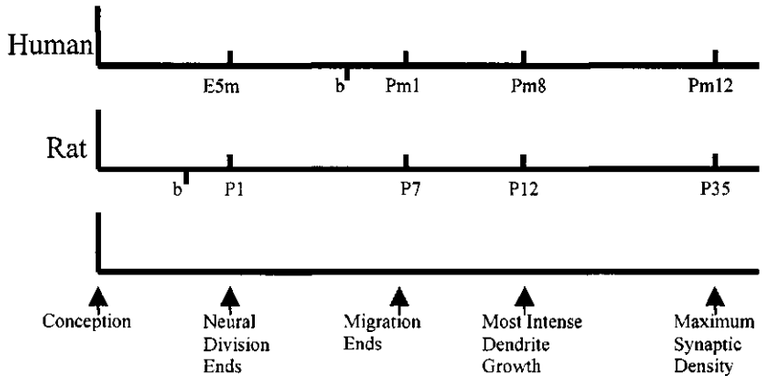

Figure 1-1. Schematic illustration of the comparable developmental ages of the brain of the rat and human. E, embryonic day; P, postnatal day, b, day of birth, Pm, postnatal month. (Kolb, 1995).

The Kennard Principle

Functional outcome after brain injury seems to be dependent on stage of development of the inflicted individual. It seems that the stage of development affects how the brain is able to recover. One theory of recovery is credited to Margaret Kennard.

In the 1930's and 40's Margaret Kennard removed the motor cortex of monkeys at various stages of development (Kennard, 1938, 1940). She noted that animals that received lesions as infants showed greater sparing of motor behaviour than animals that received their lesions as adults. Kennard's discoveries were later coined "the Kennard Principle" by Teuber (Teuber, 1975). The explanation for this phenomenon was that the developing brain is better capable of adapting, a property referred to as "plasticity." Because of the young brain's inherent plasticity, it is more apt to compensate after some types of brain injury.

Although there is considerable support for the Kennard Principle (e.g., (Payne & Lomber, 2003), numerous studies suggest that earlier brain injuries do not have a better behavioural outcome. For example, Passingham *et al.* (Passingham, Perry, & Wilkinson, 1983) showed that the age at which subjects were tested was critical for interpreting functional outcome. When tested at a young age, neonatally injured monkeys displayed better motor skills than adult operates, but their abilities deteriorated. A similar phenomenon has been described for human children (Johnson, Rose, Brooks, & Eyers, 2003). In one study, children showed significantly greater cognitive impairment 15 years after brain injury than they did immediately after the trauma (Thomsen, 1984).

Perhaps one of the shortcomings of the Kennard Principle is that it assumes that the brain is static throughout development, neglecting that the levels of plasticity vary

with the stage of development (Kolb, 1995). Further, the principle ignores the fact that development has a sequential order. Early brain damage affects the foundations of brain development. Successive stages are bound to be adversely affected.

Work by Kolb and his colleagues have shown that the Kennard principle only applies to certain types of brain injury (Kolb & Gibb, 1993; Kolb & Nonneman, 1976, 1978; Kolb, Sutherland, & Whishaw, 1983). Hemidecortication in rats for example, appears to follow the recovery pattern of Kennard's monkeys. Rats that receive hemidecortication on postnatal day three (P 3) show better functional recovery than rats that receive the same injury in adulthood. The same does not hold true for medial frontal lesions. Rats that receive medial frontal lesions at postnatal day ten (P 10) show significantly better functional recovery than rats that receive the injury at P 3.

Descending Motor Pathways

One difficulty in working with non-verbal animals is measuring the behavioural deficit and recovery from brain injury. Motor skills provide a measure independent of language. Motor skills are thought to be primarily controlled by motor cortex. Damage to this area in rats impairs their ability to reach for food and swim.

It is important to understand the anatomical connections between the motor cortex and spinal cord. In humans, the corticospinal tract is the most direct connection between the motor cortex and the spinal cord. It originates in pyramidal cells of layer V in the motor cortex. The fibres in this pathway are divided into lateral and ventral projections. The lateral portion receives projections from Brodmann's areas 6, 4, 1, 2, 3, 5, 7, and 23. It contains contralateral projections and crosses at the pyramidal decussation (Figure 1-2).

The lateral corticospinal tract terminates on the lateral intermediate zone of the dorsal and ventral horn. This system controls distal limb movement (Martin, 2003).

The ventral portion of the corticospinal tract is uncrossed. It therefore has ipsilateral projections into the spinal cord. The projections originate at Brodmann's areas 6 and 4. The ventral portion only accounts for 20% of the total fibres of the corticospinal tract. In humans, it terminates on the medial intermediate zone of the ventral horn. It controls voluntary proximal movements such as axial muscles.

The rat cortico-spinal tract contains fibres that do not project as directly as the human system (Yang & Lemon, 2003). However, lesion studies have demonstrated that motor skill and the cortico-spinal tract in the rat are sensitively connected (Iwaniuk & Whishaw, 2000; Whishaw, Gorny, & Sarna, 1998).

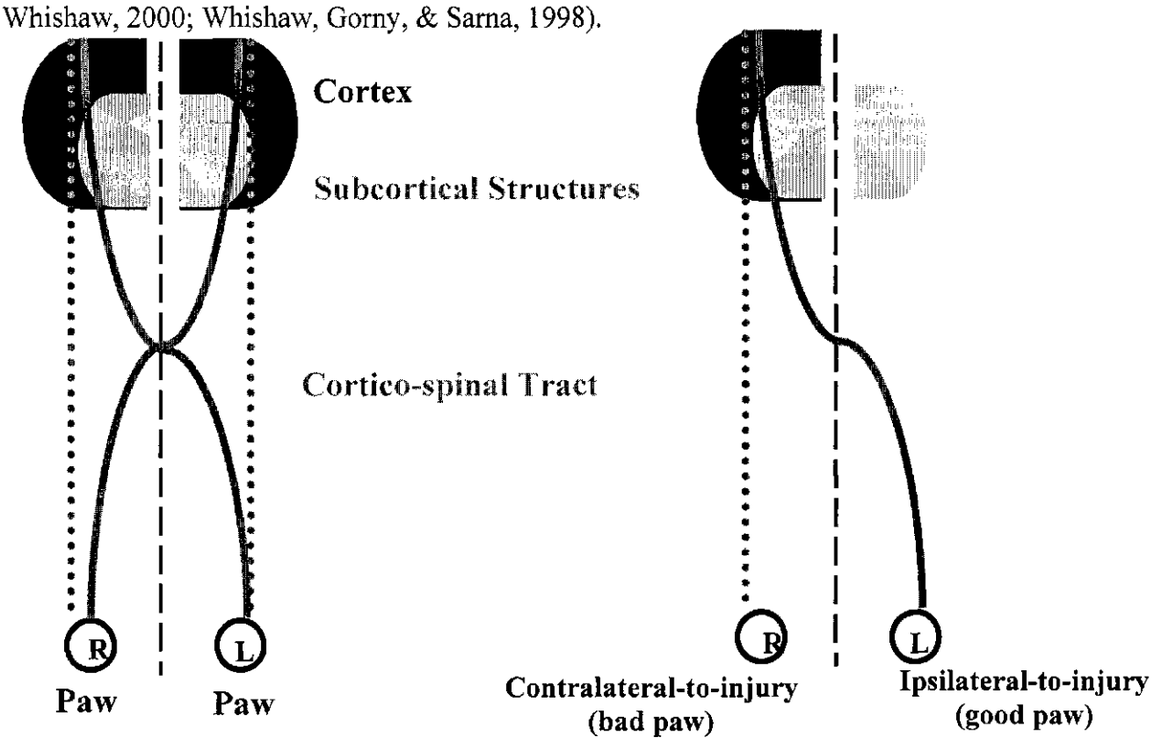

Figure 1-2. Cortico-spinal projections. On the right is a representation of what happens when the cortex is removed. The post-lesion projections are thus altered.

Hemidecortication

In some situations, cerebral structures are removed surgically in human patients. For example, certain types of epilepsy are drug resistant and require an alternate solution to control the seizures (Fusco & Vigevano, 2004). Until the 1960's, entire hemispheres of young patients were removed in order to prevent seizure activity. This practice fell out of favour after Laine, Pruvet and Ossen (Laine, Pruvot, & Osson, 1964) followed up with hemispherectomized patients and noted late complications such as intracranial bleeding. As an alternative, only the neocortex of one hemisphere in young patients was removed (Rasmussen, 1983). This procedure was less invasive and had a greater rate of success for patients.

Behavioural studies on hemispherectomy and hemidecorticated patients have shown variable results as some individuals show remarkable recovery and have been able to graduate with university degrees (Taylor, 1991). In general, however, patients show significant cognitive impairments with mean IQs in the 85 range and much lower in patients in which seizures are not controlled (Vhargha-Khadem & Polkey, 1992). A significant clinical problem therefore is to find ways to simulate better functional outcome in the typical hemidecorticated patient.

Animal models of hemidecortication also have been studied in a variety of laboratory species including monkeys, cats, and rats over the past 40 years, with the bulk of the work being done on animals with perinatal injuries. The general finding in all species is that there is significant anatomical reorganization of the connections of the remaining hemisphere after the early lesions (Castro, 1975; Rasmussen, 1983; Villablanca, 1984). Although the animals show significant cognitive and motor deficits

after these procedures, animals with perinatal hemidecortications show a much better functional outcome than adults with similar injuries.

Factors affecting functional recovery from early brain injury

After large neocortical lesions, such as hemidecortication, the brain undergoes reorganization that allows for compensation or functional recovery of cognitive and motor skills. There are two main factors that influence functional recovery from brain damage: (1) internal environment and (2) external environment.

Internal Environment. The internal environment can include anything from hormones to neurotropic factors. Gonadal hormones affect the way the brain develops and recovers from damage. The brains of male rats show a distinct asymmetry in size between hemispheres, whereas the two hemispheres of female rat brains are equal in size. Previous work by Stewart & Kolb (Stewart & Kolb, 1988) have shown that gonadectomized males have thicker cortices when compared to control males. Thus, reduced levels of testosterone because of gonadalectomy allow the left hemisphere to grow to the size of the right hemisphere. This body of work suggests that male and female rat brains are fundamentally different. It is unreasonable, therefore, to believe that the two groups will respond to brain injury in the same way.

Neurotropic factors can also affect the brain's ability to recover after damage. Neurons have the ability to anterogradely transport trophic factors. These chemicals are synthesized in the cell body, transported to axon terminals, and then released to be taken up by the postsynaptic neurons (Kolb, 1995). Basic Fibroblast Growth Factor (FGF-2) works in this way. This growth factor works globally in the brain and reduces cell death after axotomy (Cotman, Cummings, & Pike, 1993).

Fibroblast Growth Factor-2 is one of nine members in the FGF family. This growth factor is involved in cellular processes such as the mitogenic response of endothelial cells. Different isoforms of FGF-2 can be found in neurons and are important for normal development of the brain. For example, FGF-2 is important for proper cell pruning during development (Abe & Saito, 2001). This essential step in development is altered by a loss of FGF-2 and might therefore lead to abnormal cytoarchitecture (Ortega, Ittmann, Tsang, Ehrlich, & Basilico, 1998).

Fibroblast Growth Factor-2 has been associated with improved functional recovery after cortical lesions. It has been shown to support the survival of cortical projections (Catapano, Arnold, Perez, & Macklis, 2001), to enhance axonal sprouting (Ramirez et al., 1999), and to stimulate neonatal and adult brain neurogenesis (Wagner, Black, & DiCicco-Bloom, 1999). Alternately, blocking FGF-2 impairs functional recovery after motor cortex lesions in rats (Rowntree & Kolb, 1997). Previous studies have also shown that subcutaneous injections of FGF-2 can lead to a regrowth of tissue from a motor cortex lesion (Monfils et al., 2004; Monfils et al., 2005).

External Environment. Experience, or the external environment, can be defined as anything from tactile stimulation from the mother to an enriched environment. An enriched environment is an enlarged living situation where groups of rats can be housed (Figure 1-3). The animals are exposed to a variety of toys, foods, and other rats. Rats that have been housed in an enriched environment show increased cortical thickness, increased dendritic branching, and increased numbers of synapses in the cortex (Kolb & Gibb, 1991). Environmental enrichment remodels cortical connections even in control animals.

Environmental enrichment has been used as a treatment after neonatal and adult hemidecortication (Whishaw, Sutherland, Kolb, & Becker, 1986). These animals were then tested in a cognitive task. Animals that received an adult hemidecortication and were exposed to an enriched environment showed significant improvement at this cognitive test. Furthermore, environmental enrichment has been shown to improve the functional recovery of frontal lesions and motor cortex lesions (Kolb & Elliott, 1987; Kolb & Gibb, 1991).

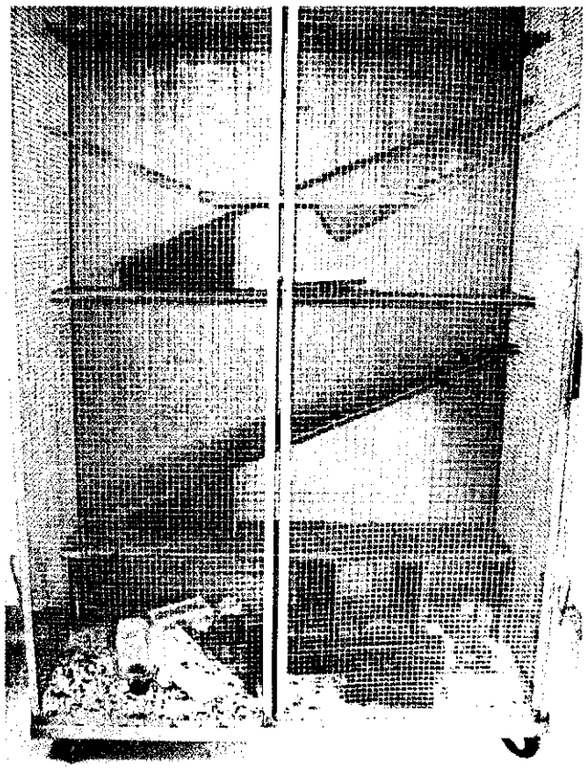

Figure 1-3. An enriched environment for rats. Courtesy of R.L.Gibb.

Because animals are housed in large numbers in complex housing their skin and vibrissae are continually stimulated. Tactile stimulation is an important element of environmental enrichment. Because pre-weanling rats cannot easily move about, an enriched environment is not a convenient or useful treatment. It is possible, however, to focus on the tactile stimulation element and use this for neonatal animals.

Tactile stimulation has been shown to stimulate growth in premature infants (Field et al., 1986) and newborn rats (Schanberg & Field, 1987). Recently, Robbin Gibb (Gibb, 2001) has shown that tactile stimulation aids in recovery of function after neonatal frontal lesion.

Figure 1-4. The Tactile Stimulation Treatment.

Objectives of Present Study

The goal of this thesis is to investigate the behavioural effects of large neocortical lesions during different stages of development and to attempt explore treatments that have previously been found to be effective in focal lesions in laboratory rats. These

procedures will also allow for the assessment of neurophysiological and anatomical properties of the neocortex on the non-injured hemisphere. These main goals can be achieved with three experiments: (1) assess the behavioural and anatomical outcome of rats that received a hemidecortication either as a neonate (postnatal day ten) or adult (postnatal day 90); (2) assess whether tactile stimulation improves the functional outcome in adulthood of animals that received a lesion on postnatal day ten (P 10); (3) assess the functional outcome in adulthood of rats that received P 10 hemidecortication and subcutaneous injections of FGF-2.

Experiment 1. Hemidecortication was performed in rats on either P 10 or in adulthood (P 90). At P 120 the animals were then tested on a battery of motor skill measures. This included the tray reaching task, the single pellet reaching task, the forepaw asymmetry task, and the sunflower seed consumption task (these will be discussed in detail below). The motor cortices were then mapped using intracortical microstimulation (ICMS) or stained using the Golgi-Cox method to assess the neurophysiological and neuroanatomical properties of the non-injured hemisphere.

Experiment 2. The tactile stimulation treatment was administered to rats with P 10 hemidecortication. In adulthood, the animals were tested on the same motor behaviours as well as the Morris water task to assess the cognitive spatial abilities of these animals. The objective of this experiment was to improve functional outcome after P 10 hemidecortication. The tactile stimulation treatment has previously been shown to improve the functional outcome after small focal lesions of the neocortex (Gibb, 2001). This was the first time it will be attempted for large neocortical lesions.

Treated animals received tactile stimulation for 15 minutes, three times a day. The treatment began the day after surgery (postnatal day eleven) and continued until weaning at postnatal day 21. The tactile stimulation was done with a standard size Clinique blush brush (Figure 1-4).

Experiment 3. FGF-2 injections were given to rats with P 10 hemidecortication. The motor and cognitive tasks from the second experiment were repeated. The objective of this experiment was to see if a growth factor that is known to aid in recovery of function after focal lesions can help animals that had received hemidecortication.

Postnatal day ten hemidecorticate animals received subcutaneous injections of 10μg/kg of FGF-2. The treatment began on postnatal day eleven and continued daily for one week.

The results and implications of each experiment will be discussed throughout the thesis and summarized in the final chapter.

Behavioural Tasks

The following behaviours will be used to measure behavioural outcome in each of the experiments mentioned above.

Morris Water Task. In 1981, Richard Morris developed a task that allowed researchers to study the cognitive abilities of rats (Morris, Garrud, Rawlins, & O'Keefe, 1982; Sutherland, Kolb, & Whishaw, 1982). The apparatus involves a large, circular pool that is filled with water (Figure 1-5). The water is rendered opaque with skim milk powder. In one version of this task, rats locate a hidden platform by learning the location of static extra-maze cues. Rats are quick to learn this task. The animal's abilities can be assessed in several ways. For example, latency to reach the platform or swim path length can be two accurate measures. Animals with certain types of cortical injury appear to have deficits at this task. It is important to note that the deficits are not due to swimming abilities but rather to the ability to learn the location of, and navigate to, the platform.

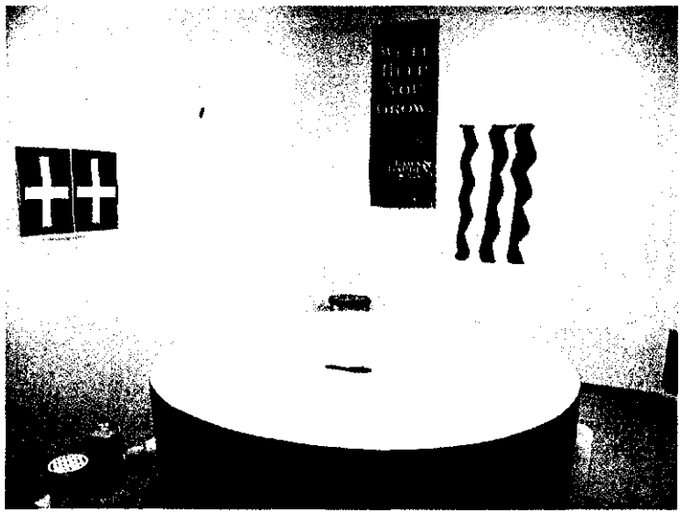

Figure 1-5. The Morris Water Task.

Tray Reaching Task. Whishaw *et al.* (Whishaw, O'Connor, & Dunnett, 1986; Whishaw & Pellis, 1990) developed two tasks to reaching and grasping for food. In the tray reaching task a rat is trained to reach through metal bars to retrieve chicken feed from a tray at the front of the cage (Figure 1-6). Performance is measured by the success of the animal to retrieve and consume food.

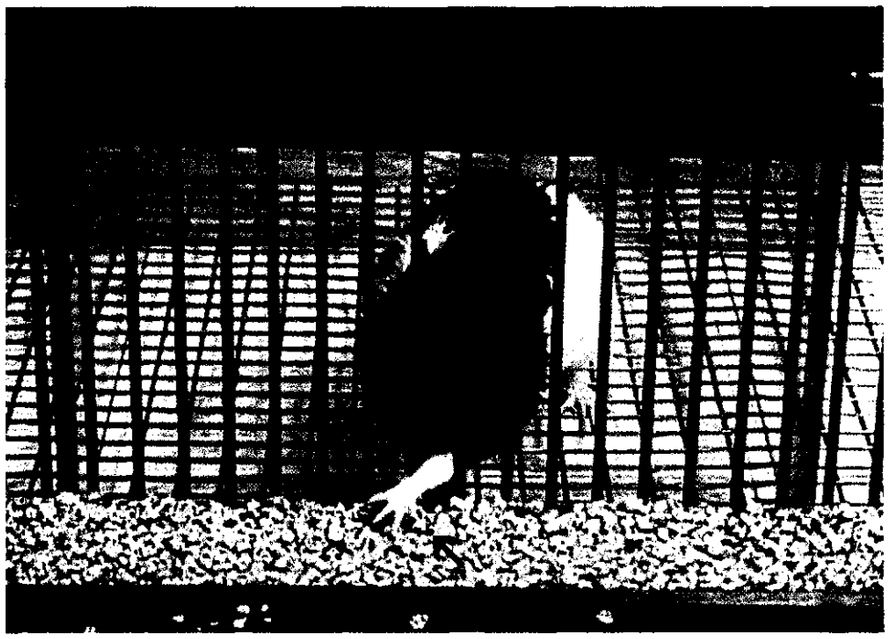

Figure 1-6. Tray Reaching Task. (Courtesy of I.Q. Whishaw.)

Single Pellet Reaching. This task requires the rat to reach through a narrow opening at the front of a Plexiglas box and retrieve a single food pellet (Figure 1-7). This task requires more precision on the part of the animal due to the narrow opening and the size of the pellet. This task provides additional insight into the organization of skilled movements and the nature of each animal's deficits because movements of reaching can be analyzed from video records using frame-by-frame analysis.

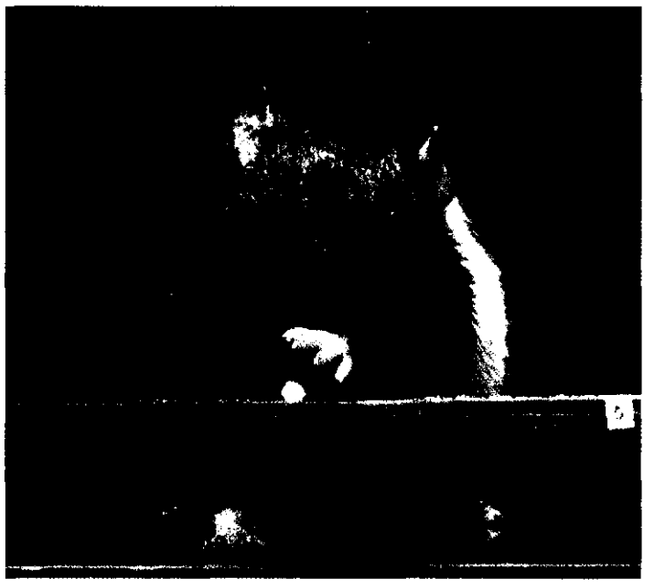

Figure 1-7. The Single Pellet Reaching Task. (Courtesy of O.A.Gharbawie.)

Forepaw Asymmetry Task. This task takes advantage of a rat's natural tendency to explore a novel environment (Schallert, Kozlowski, Humm, & Cocke, 1997). Rats are placed in a large Plexiglas cylinder and allowed to explore for three minutes (Figure 1-8). The rats rear onto their hindlimbs and use their forepaws to contact the vertical surface of the cylinder. Asymmetries in forepaw contact can provide an index of lateralized brain injury. For example, animals with unilateral motor cortex damage will favour their unimpaired forelimb when supporting their weight around the cylinder wall.

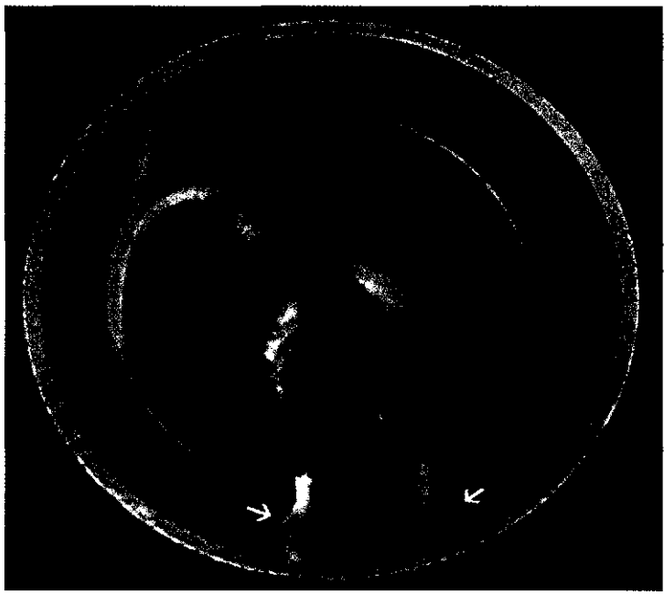

Figure 1-8. The Cylinder Task. (Courtesy of O.A. Gharbawie.)

Sunflower Seed Consumption Task. In this task an animal must successfully open and consume five sunflower seeds (Figure 1-9). The time that the rats spend manipulating, opening and consuming the seeds can be recorded. Animals with cortical lesions take longer to perform this task.

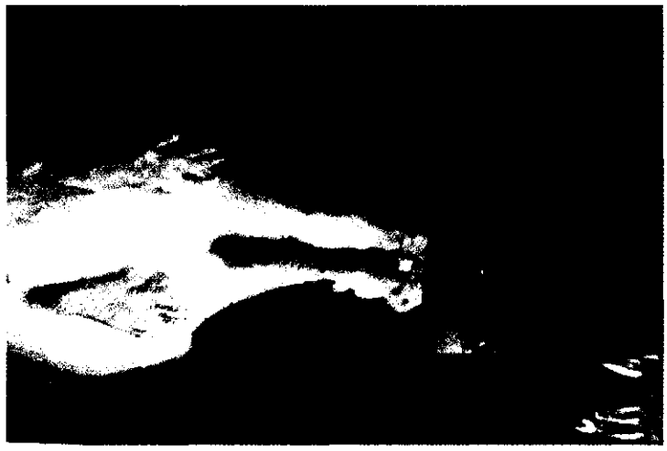

Figure 1-9. The Sunflower Seed Task. (Courtesy of C.L.Gonzalez.)

Anatomical Measures

Golgi-Cox method. Camillo Golgi developed this technique in 1873. Early investigators, such as Ramon y Cajal, used the Golgi technique to define structural features of brain architecture (DeFelipe & Jones, 1988). Only a small percentage of neurons (1-5 %) are randomly stained using this procedure. Because the cells are stained completely (Figure 1-10), it is possible to draw individual neurons and quantify the amount of dendritic space available.

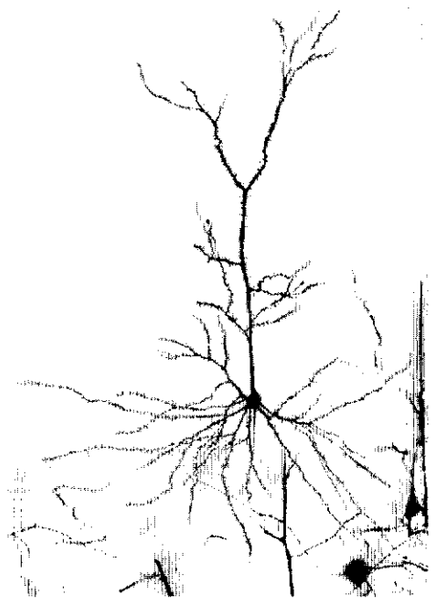

Figure 1-10. Layer III pyramidal cell stained with Golgi-Cox. (Courtesy of G. Gorny.)

Intracortical Microstimulation (ICMS). In this process, movement representations are defined as the cortical loci from which movements about individual joints, or specific muscle groups can be evoked. An electrode is lowered into layer V of the motor cortex where the somata of corticospinal cells reside. A small amount of electrical current is delivered in a brief train. This results in the activation of relatively small groups of corticospinal cells, which in turn evoke movements of specific body regions (Jankowska, Padel, & Tanaka, 1975). The movement evoked at the lowest possible current level is defined and coded on a digital photograph of the surface vasculature at each site of stimulation (Figure 1-11). The final product is a mosaic map of the motor cortex with the representations of each muscle group

Figure 1-11. The Intracortical Microstimulation Technique.

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2. EXPLORING AGE AS A FACTOR OF RECOVERY AFTER HEMIDECORTICATION: A BEHAVIORAL, ANATOMICAL, AND NEUROPHYSIOLOGICAL STUDY IN RATS

ABSTRACT

Hemidecortication is a well-known treatment for severe unilateral cerebral dysfunction and is associated with significant anatomical reorganization. The objective of the present experiment was to analyze the sensorimotor forelimb function of rats after infant or adult hemidecorticate by utilizing sophisticated behavioural analyses. Rats were given hemidecortications either on postnatal day 10 (P 10) or 90 (P 90). Both groups were then tested on a number of behavioural tasks (skilled reaching, forelimb placing during spontaneous vertical exploration, and the sunflower seed task) beginning at P 120. In some of the female animals, topographic movement representations were derived in the hemisphere contralateral to lesion using Intracortical Microstimulation (ICMS). The brains of the male animals were prepared for Golgi-Cox staining and subsequent analysis of dendritic arborisation and spine density. Three main findings came out of this experiment. 1) Both groups of hemidecorticate animals were impaired when tested on the motor tasks in this experiment but the impairments were qualitatively different in the neonatal and adult operates. For example, the P 10 hemidecorticate animals displayed mirror movements. 2) Hemidecortication in adults, but not P 10 neonates, led to increased dendritic arborisation of Layer III pyramidal cells in the parietal cortex. 3) P 10 hemidecortication altered the details of the ICMS-delinated motor maps. From this study it was concluded that, depending on the time of injury, the central nervous system compensates for damage in different ways.

INTRODUCTION

There appear to be critical periods in which the brains of mammals are more "plastic." That is, the brain shows a greater potential for change during these periods. This change is presumed to underlie better functional outcome after brain injury. For example, Margaret Kennard noted that monkey neonates showed greater behavioural sparing after motor cortex injury than adults with similar injuries (Kennard, 1942). Kennard's experiments led to an idea that earlier was better when it came to the age at which animals sustained brain injury.

Although there is considerable support for this idea both anecdotally and in the research literature (e.g., Payne & Lomber, 2003), numerous studies have suggested that this conclusion is too simple (Kolb, 1987; Villablanca, Hovda, Jackson, & Gayek, 1993). For example, Kolb demonstrated that rats that receive a bilateral medial frontal lesion in the first week of life are severely impaired when compared to rats that receive the same lesion in their second week of life (Kolb, 1987). Later experiments looking at the effects of motor, parietal, visual, temporal, orbital frontal, and posterior cingulate cortex found similar results (e.g., Kolb, 1995). In contrast, however, damage during the second week of life allowed significant functional recovery as Kennard had found in her infant monkeys. It thus appears that the precise timing of brain injury is important in understanding the functional outcomes of perinatal injury.

One complication of the studies of both Kennard and later by Kolb and others is that these studies were largely done on animals with focal lesions. It appears that the "rules" for functional recovery after perinatal injury may be quite different for more extensive injuries such as hemidecortication.

Hemidecortication is a well-known treatment for severe unilateral cerebral dysfunction in children and is associated with significant anatomical reorganization in laboratory animals (e.g., Castro, 1975). Recovery of function after hemidecortication is not well understood, however. Previous studies have shown that rats that receive hemidecortication in their first week of life have a thicker cortex in the remaining hemisphere in adulthood than adults that receive the same lesion (Kolb & Tomie, 1988). These authors also reported that hemidecortication early in life versus hemidecortication in adulthood leads to better functional outcome by the neonatal operates on tasks such as beam walking and forepaw inhibition. They also noted that with some behavioural tasks, such as the Morris water task, infant hemidecortication afforded no advantage. The problem with these studies was that the behavioural measures did not include a sensitive analysis of motor function as only gross motor function was studied.

The objective of the present experiment was to analyse the sensorimotor forelimb function of infant and adult hemidecorticate rats by utilizing more extensive behavioural and electrophysiological analyses than were used in the earlier experiments. The expectation was that the P10 and P90 animals might show qualitative differences in the pattern of motor recovery and that such differences would be related to both motor representations and the general morphology of dendritic fields in the intact hemisphere. Rats were given hemidecortications either on postnatal day 10 (P 10) or 90 (P 90). Both groups were then tested on a number of behavioural tasks beginning at P 120. In a portion of the female animals, topographic movement representations were derived in the hemisphere contralateral to lesion using intracortical microstimulation (ICMS). The

brains of the male animals were prepared for Golgi-Cox staining and subsequent analysis of dendritic arborisation and spine density.

METHODS

Subjects

Subjects were 31 male and 28 female Long-Evans hooded rats. All animals were raised in the University of Lethbridge vivarium and were housed in groups of 5 in hanging plastic tubs. The colony room was controlled on a 12 hr light: 12 hr dark cycle (lights on 07.30-19.30 hr) and the temperature maintained at 22°C. Experiments were conducted according to standards set by Canadian Council on Animal Care and approved by the University of Lethbridge animal care committee.

Surgical Procedures

The animals were split into three groups: a sham control group (composed of 18 day 10 shams and two day 90 shams) was done with each of the hemidecorticated groups, hemidecortication at postnatal day ten (P 10), and hemidecortication at P 90 (in adulthood).

At postnatal day ten, rats (26 male and 23 female) were anesthetised by cooling in a Thermatron cooling chamber until the body temperatures were in the range of 18-20°C. Surgery was performed using suction by pipette. The surgeon performed these operations with the aid of a surgical microscope (Kolb, Sutherland, & Whishaw, 1983). Rats received either a complete left hemisphere decortication or a scalp incision (sham controls). Following surgery, the pups were warmed up by hand and then placed on a heating pad until all of the animals were moving. They were then returned to the dam.

At P 90, five male and five female rats were anesthetised with isoflurane. Hemidecortication was performed as described in Kolb, Gibb, & van der Kooy, (1992). Four rats of either sex received a complete left hemisphere decortication. The remaining two rats were anesthetised and received a sham scalp incision. Rats were fed mash (a combination of powdered food pellets and water) for one week or until they could eat hard rat chow.

Food restriction

Subjects were kept on a restricted food regime. Each animal received 20g of food per day after the testing session was complete. Their body weight was maintained at ≈ 90-95% until the completion of the behavioural testing.

Tray reaching

The animals were placed in a test cage (10x18x10 cm high) with floor and fronts constructed of 2mm bars, 9 mm apart edge to edge. A 4 cm wide and 1 cm deep tray, containing chicken feed pellets, was mounted in the front of each box (Figure 1-6).

The rats were food deprived to 90% of their normal body weight for testing. The rats were required to extend a forelimb through the gap in the bars, grasp and retract the food and eat it. Animals were tested every day for three weeks in the tray reaching apparatus. They were allowed to reach with either paw. At the end of each test week, rats were filmed for 5 mins and the tapes were scored for numbers of attempted and successful reaches. The percent success was calculated as follows:

(Successful reaches/ (successful reaches + attempts)) x 100%

This was calculated for each week and then averaged.

Single Pellet reaching

The single pellet reaching boxes were made out of clear Plexiglas with the dimensions 45cm deep x 14cm wide x 35cm high. In the centre of the front wall was a 1 cm wide slit which extended from 2 cm above the floor to 15 cm above (see Figure 1-7). In front on the slit on the outside of the box there was a 2 cm wide shelf. The shelf was placed 3 cm above the floor. Two indentations were made on the shelf 2 cm from the inside of the wall. The indentations were centred to the slit so the rats could easily reach them. Food pellets (45 mg Rodent Chow food pellets, Bioserve) were placed in the indentation opposite to the rats' preferred reaching paw.

The animals were trained as adults for 2 weeks and then tested for nine days on the single pellet reaching task. They were presented with 20 pellets in each testing session. A successful reach was defined as the pellet being retrieved from the platform and eaten by the animal. At times the rat would reach and miss the pellet but repeat the reaching movement until it finally retrieved the pellet. Each reaching movement was counted as an attempt. The performance of each animal was analyzed in two ways. First, the number of successful reaches out of twenty trials was analyzed. Second, the accuracy of each reach was analysed. This was calculated as follows:

Number of attempts/ # of pellet successfully retrieved

An attempt was classified as the motion of reaching for the pellet. A score of 1 is perfect, as it would take one attempt to retrieve one pellet.

Forepaw Asymmetry

Forelimb use during spontaneous exploration was examined by placing rats in a transparent cylinder 20 cm in diameter and 30 cm in height (Schallert, Kozlowski,

Humm, & Cocke, 1997). A mirror was placed beneath the cylinder to allow the activity of the rat to be videotaped. For the video analysis, each forepaw contact with the cylinder wall was counted (Figure 1-8). The asymmetry score [i.e. contralateral forelimb/ (contralateral + ipsilateral)] of forepaw use was calculated for both the first touch of any bout of exploration and the total amount of touches for the testing period. Animals were individually placed in the cylinder for a single, three minute testing session.

Sunflower Seed Task

Five sunflowers seeds were placed in the corner of a clear Plexiglas box (50 x 50 x 50cm). The rat was allowed to explore the box until it discovered the seeds. Rats began by manipulating the seeds into their preferred position before removing the shell (Figure 1-8). The animal would then eat all five seeds in succession. The total amount of time which the animal spent manipulating, opening, and consuming the seeds was recorded. Animals were trained for one day and tested on the second and third day.

Anatomical Procedures

The animals were separated by sex for the anatomical studies. Only male brains were stained with Golgi-Cox and only female brains were mapped with ICMS.

Male animals were given an overdose of sodium pentobarbital and perfused intracardially with 0.9% saline. The brains were removed and weighed before being submerged in ~20mL of Golgi-Cox solution. The brains were left in this condition for 14 days and then placed in a 30% sucrose solution for two days. The brains were cut on a vibratome at 200μm and developed using the procedure described by Gibb & Kolb (1998). Layer III pyramidal cells were traced in Zilles' parietal area 1 using camera lucida at 250X magnification. Dendritic length and branching were measured on these

drawings. Branch order analysis was done according to Coleman & Riesen (1968). Branch length also was measured indirectly by Sholl analysis (1956).

Cortical Thickness

Cortical thickness was measured on the Golgi-Cox stained brains. Only males were used for this analysis. The dimensions were taken on a Zeiss 2 POL projector set at a magnification of 10x. Measurements were taken at three different points at each of five planes corresponding to Zilles' levels +2.2, -0.3, -2.3, -4.8, and –6.3 relative to bregma, (Stewart & Kolb, 1988). The planes were chosen for their correspondence to the anterior tip of the caudate-putamen, the middle of the anterior commisure, the rostral tip of Ammon's horn, the posterior commisure, and the posterior tip of the hippocampus. The multiple measurements from each plane were averaged and then all five planes were averaged again.

Intracortical Microstimulation

Detailed maps of the forelimb representations within the motor cortex were generated using intracortical microstimulation (ICMS) (Kleim et al., 2002). Eight female animals (4 P 10 hemidecorticates and 4 sham operated cohorts) were food deprived for 16 hours prior to the ICMS session. The subjects were anaesthetized with ketamine hydrochloride (70mg/kg i.p.) and xylazine (5 mg/kg i.m.). They also received xylazine (0.02 mg/kg i.p.) and ketamine as needed. A craniotomy was performed over the motor cortex of the right hemisphere. The cisterna magnum was punctured to reduce edema before retracting the dura. The cortex was then covered with warm silicon oil (37°C). A glass microelectrode controlled by hydraulic microdrive was used to penetrate the cortical layer V (a depth of ~1550 μm). The stimulation sites were spaced apart at a

distance of 375μm. Stimulation consisted of thirteen, 200μs cathodal pulses delivered at 350 Hz from an electrically isolated stimulation circuit. Animals were maintained in a prone position, with constant limb support. The minimum threshold required to elicit a movement was recorded for each penetration site. If no movement was detected at ≤ 60 μA the site was recorded as non-responsive. Breathing rate was monitored to assess the animal's responsiveness. Forelimb movements were classified as either distal (wrist/digit) or proximal (elbow/ shoulder) and representational maps were generated from the pattern of electrode penetrations. An image analysis program (CANVAS v. 3.5) was used to calculate the extent of caudal and rostral forelimb area (CFA & RFA; (Remple, Bruneau, VandenBerg, Goertzen, & Kleim, 2001).

Statistical Analysis

Analyses of variance (ANOVA) were used for all measurements and Fisher's LSDs ($p < 0.05$ or less) were used for *post hoc* evaluations. Because no sex differences could be measured the data were collapsed across this factor.

Timeline

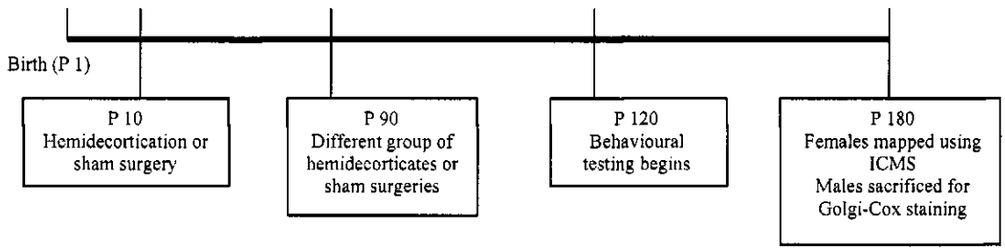

Figure 2-1. A timeline of the methods performed in this experiment. P, postnatal day.

RESULTS

Surgical Recovery

Shortly after surgery, the P 10 hemidecorticate animals could be seen suckling from their dam and grouping together. Thus, the behaviour of these animals was typical for rats of this age and there was no obvious difference between shams and hemidecorticates.

One female P 90 animal died within an hour of surgery. Within 12hrs, the remaining animals were able to consume the mash made of water and powdered pellets. None of the animals used the paw contralateral to the injured hemisphere (i.e., they were hemiplegic). Over the first post-operative week, the hemidecorticate animals lost some weight, but gained it back before behavioural testing began (data not shown).

BEHAVIOURAL RESULTS

Tray Reaching

Rats were tested for three weeks in the tray reaching apparatus. They were allowed to reach with either paw. All hemidecorticated rats favoured their ipsilateral paw when reaching for the food. The hemidecorticate animals appeared extremely anxious during their first week of testing. Any movement or noise would stop them from attempting to retrieve the food. An ANOVA revealed that both of the hemidecorticate groups were impaired at this task, even when using the limb contralateral to the intact hemisphere [$F(2,56) = 9.851, p < 0.01$].

A Fisher's LSD displayed a significant difference between the control group and the P 10 hemidecorticate group ($p < 0.01$). There was also a strong trend between the

control and adult (P 90) hemidecorticate group ($p = 0.06$). However, the two hemidecorticate groups did not differ (Figure 2-2).

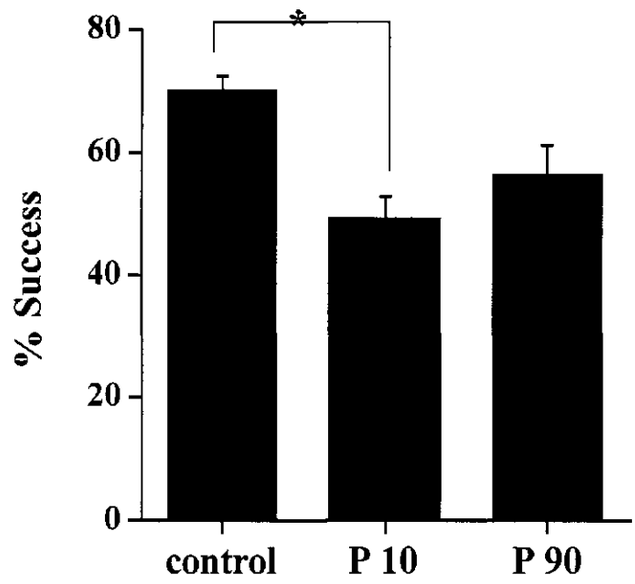

Figure 2-2. Tray Reaching Task. Mean success of retrieving food pellets. The results reflect the success over three weeks for each group. The P 10 hemidecorticate group was impaired in comparison to the control animals. The P 90 hemidecorticates did not differ from either the control or P 10 group. (P 10) Neonatal hemidecorticate, (P 90) Adult hemidecorticate. (* < 0.05)

Single Pellet Reaching Task

Both groups of hemidecorticate rats displayed qualitative impairments in skilled reaching. A control animal typically reaches for food with one paw while the non-reaching paw remains on the floor of the apparatus during the entire reaching behaviour. In animals that received hemidecortication at P 90, the contralateral limb did not appear to be functioning (Figure 2-2). It remained on the floor during the single pellet reaching task. Unlike a control animal, however, the P 90 hemidecorticates did not lift the non-reaching paw when consuming the food pellet.

The P 10 animals displayed a very different type of compensatory technique. These rats would reach with both paws simultaneously, essentially making mirror movements (Figure 2-3). This behaviour was not restricted to the single pellet task but was also observed in the cylinder and sunflower tasks (see below).

In this task animals were allowed to reach for a total of twenty pellets (Figure 2-4A.). A one-way ANOVA showed a significant main effect of the lesion [$F(2,34) = 6.460, p < 0.01$]. Fisher's LSDs showed a significant difference between the control and P 10 groups ($p < 0.01$) but the P 90 group showed only a nonsignificant trend to be impaired ($p = 0.0939$).

The animals that received their lesion at P 90 were also the most accurate. There did appear to be a main effect of lesion [$F(2,34) = 3.862, p < 0.05$]. The Fisher's LSDs displayed a significant difference between the control and P 90 group ($p < 0.05$) and the P 10 and P 90 group ($p < 0.01$).

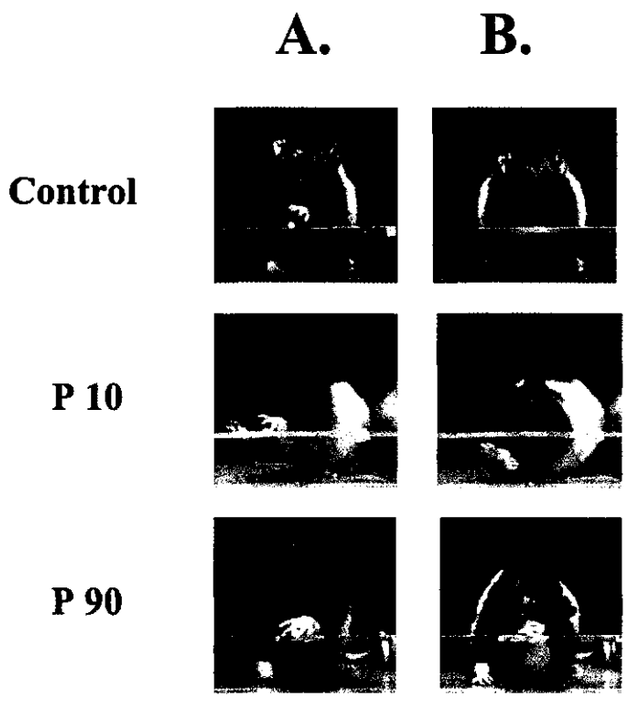

Figure 2-3. Examples of compensatory techniques used by P10 and P 90 hemidecorticate animals. A. Shows the reaching technique. B. Shows the animals consuming the pellet. (P 10) Neonatal hemidecorticate (P 90) Adult hemidecorticate.

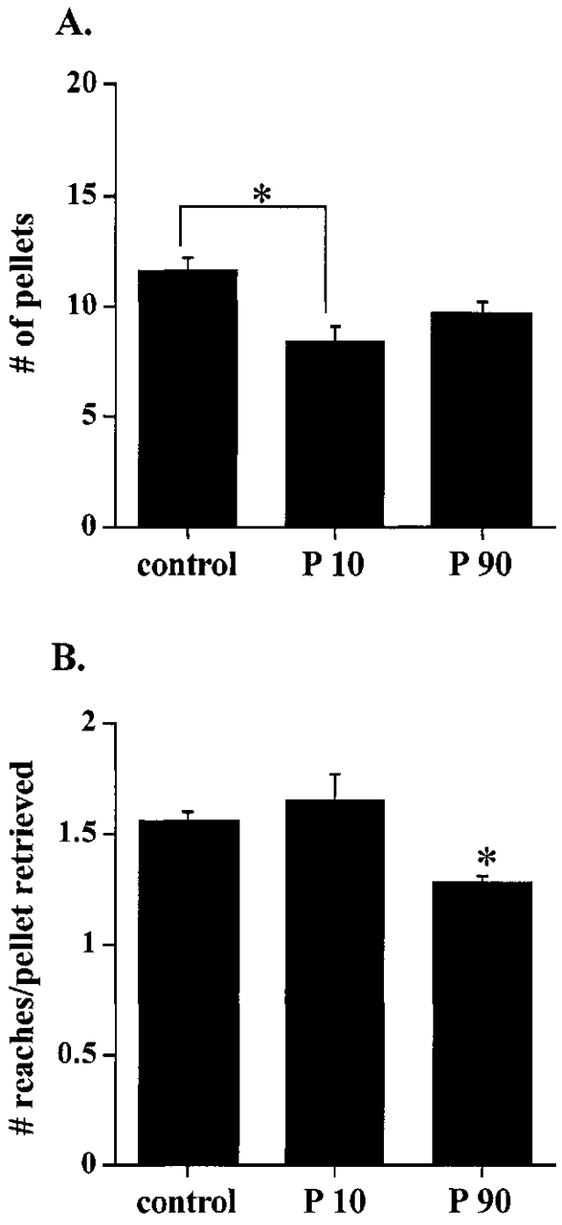

Figure 2-4. Single Pellet Reaching Task. The abilities of rats to retrieve pellets in the single pellet reaching task were measured by A. Total number of pellets (mean ± SEM) retrieved and B. Accuracy measured by number of reaches / pellet retrieved. The P 10 hemidecorticate animals were the most impaired at this task and retrieved the least number of pellets and the P 90 hemidecorticates were significantly more accurate. (P 10) Neonatal hemidecorticate (P 90) Adult hemidecorticate. (* < 0.05)

Forepaw Asymmetry

When exploring a novel environment, control rats show equal use of both forepaws to support themselves as they rear (Schallert et al., 1997). Both hemidecorticate groups showed an asymmetry in paw use at this task [F (2,45) = 7.580, $p < 0.01$] (Figure 2-5). Thus, both hemidecorticate groups were much more reliant on their ipsilateral paw than controls ($p's < 0.01$). The effect was especially dramatic in the adult hemidecorticates as the contralateral paw of the P 90 hemidecorticate animals was rarely lifted and remained predominantly at the side of the torso.

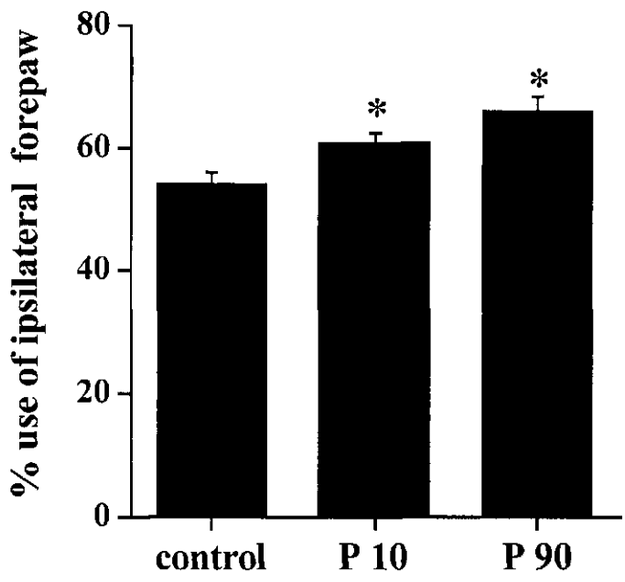

Figure 2-5. Forepaw Asymmetry Task. Relative use (mean ± SEM) of the ipsilateral forepaw in the exploration of a cylinder. Both hemidecorticate groups were more asymmetrical than the control animals. (P 10) Neonatal hemidecorticate (P 90) Adult hemidecorticate. (* < 0.05)

Sunflower Seed Consumption Task

The sunflower seed consumption task measures the amount of time it takes an animal to eat five seeds in succession (Figure 2-6). It requires the animals to use both paws at the same time. There was a main effect of the lesion [F (1,42) = 18.481, $p <$ 0.0001] and all groups differed in the manner they consumed the seeds in this task. The P10 group appeared to have some difficulty opening their digits, but the P 90 group hardly opened their digits at all on the contralateral paw. The P 10 hemidecorticate animals were significantly impaired when compared to the control group ($p < 0.01$). The P 10 group was significantly better than the P 90 group however ($p < 0.01$). The P 90 hemidecorticate group was also impaired when compared with the control group ($p <$ 0.0001).

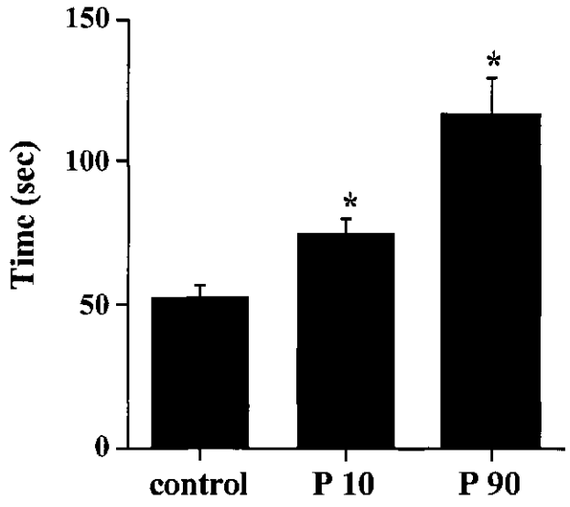

Figure 2-6. Sunflower Seed consumption Task. Mean time (mean ± SEM) to consume five sunflower seeds. All groups were significantly different than each other (* < 0.05). (P 10) Neonatal hemidecorticate, (P 90) Adult hemidecorticate.

ANATOMICAL RESULTS

Gross morphology

Both lesion groups had large removals that included virtually all of the neocortex of one hemisphere (Figure 2-6). The hippocampus appeared to be largely intact in all animals. Some anterior midline tissue was spared in the P10s but not in the P90s. In addition, the olfactory bulbs of the P10 hemidecorticates appeared visibly smaller on the lesion than on the contralateral hemisphere. Finally, the P90 brains had more visible scar tissue than the P10 brains.

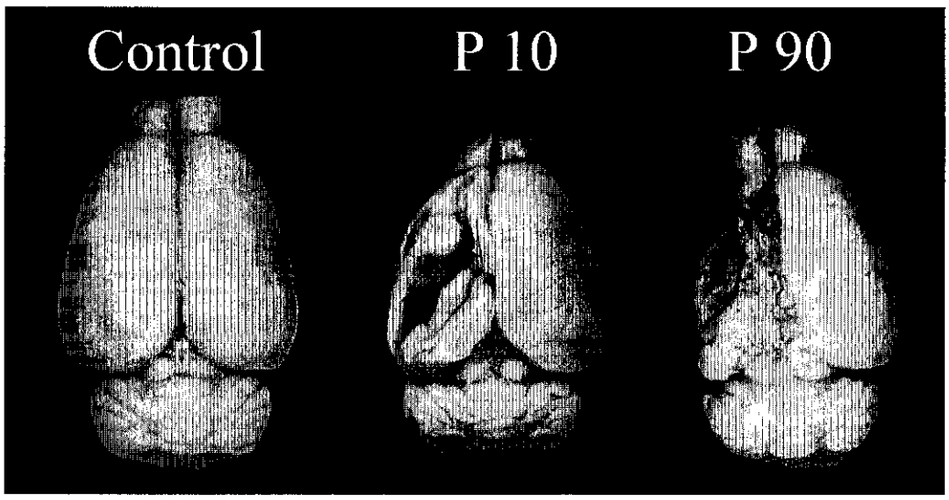

Figure 2-7. Representative brains from each experimental group. All brains are from male subjects. (P 10) neonatal hemidecorticate, (P 90) adult hemidecorticate.

Male Brain Weight

The hemidecorticated male brains were lighter than the male control brains (Figure 2-8). A simple ANOVA showed a main effect of lesion [F (2,32) = 22.796, $p < 0.0001$]. The Fisher's LSD showed that the control brains were significantly heavier ($p < 0.05$) than the hemidecorticate groups which did not differ from one another.

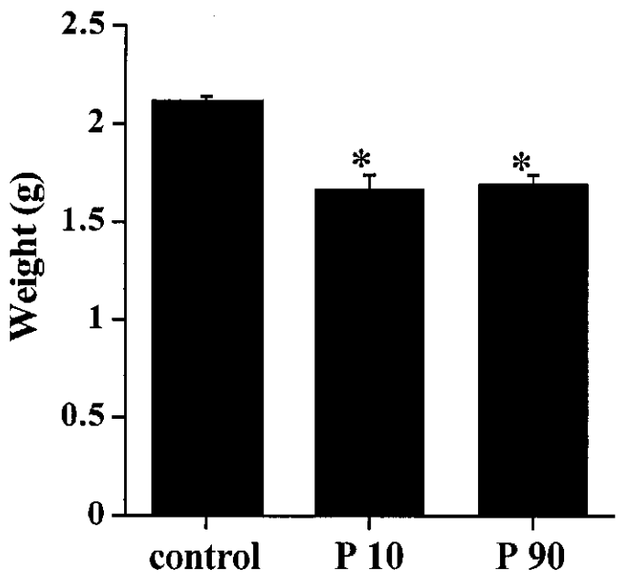

Figure 2-8. Mean brain weight of male rats. (P 10) Neonatal hemidecorticate (P 90) Adult hemidecorticate. (* < 0.05)

Cortical Thickness

Cortical thickness was measured throughout the brain. A one-way ANOVA showed a marginal main effect of lesion [$F(2,9) = 4.032$, $p = 0.0562$]. The P 10 animals did not show a decrease in cortical thickness when compared to the control animals ($p > 0.9$). The cortical thickness of the P 90 hemidecorticates was significantly thinner than both the control ($p < 0.05$) and P 10 groups ($p < 0.05$). Figure 2-9 shows the mean cortical thickness throughout the brain for each of the groups.

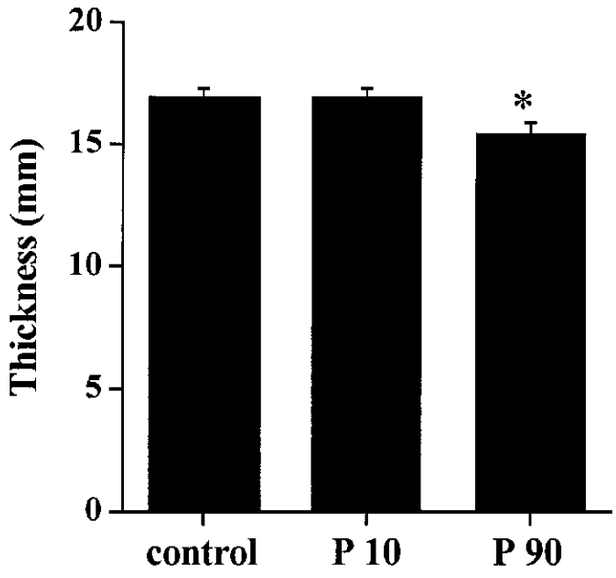

Figure 2-9. Mean cortical thickness throughout the brain. Only male brains were measured. The measurement recorded reflects the thickness at 20x magnification. The P 90 hemidecorticate group has a thinner cortex than both the control and P 10 hemidecorticate group. (P 10) Neonatal hemidecorticate (P 90) Adult hemidecorticate. (* < 0.05)

Golgi-Cox Analysis

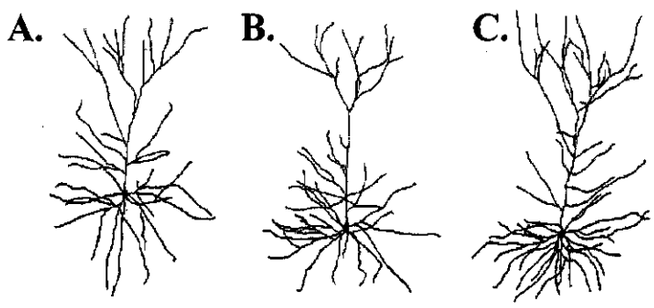

Figure 2-10. Represenative layer III parietal cells from each group. Cells were drawn on the contralateral hemisphere to the lesion. The cell from the P 90 hemidecorticate group was both larger and more complex than either of the other two cells. A. control; B. P 10 hemidecorticate; C. P 90 hemidecorticate.

The animals that received their injury in adulthood (P 90) showed larger, more complex pyramidal cells than the animals in either of the other groups. Cells were drawn in layer III parietal cortex in the hemisphere contralateral to injury. Figure 2-11 displays the measurements from the apical tree of the pyramidal cells. There was a significant difference in the branch order analysis of the apical tree [$F(2,11) = 4.542, p < 0.05$]. The cells from the P 90 hemidecorticate animals showed significantly more apical branching than both the control ($p < 0.05$) and the P 10 group ($p < 0.05$). Apical dendritic length was also different between the groups [$F(2,11) = 5.087, p = 0.0273$]. The P 90 hemidecorticates showed longer apical dendrites than both the P 90 group ($p < 0.05$) and the P 10 group ($p < 0.01$).

Figure 2-12 shows the measurements from the basilar tree of the pyramidal cells. The groups showed a difference in the branch order of the basilar tree [$F(2,11) = 3.727, p = 0.05$]. The cells from P 90 hemidecorticate animals were again more complex in the basilar tree than the control group ($p < 0.05$) but failed to differ from the P10 animals. There was, however, a significant difference between the lengths of the basilar dendrites of all groups. The P 90 hemidecorticate animals had longer dendrites than both the control ($p < 0.05$) and the P 10 group ($p < 0.05$).

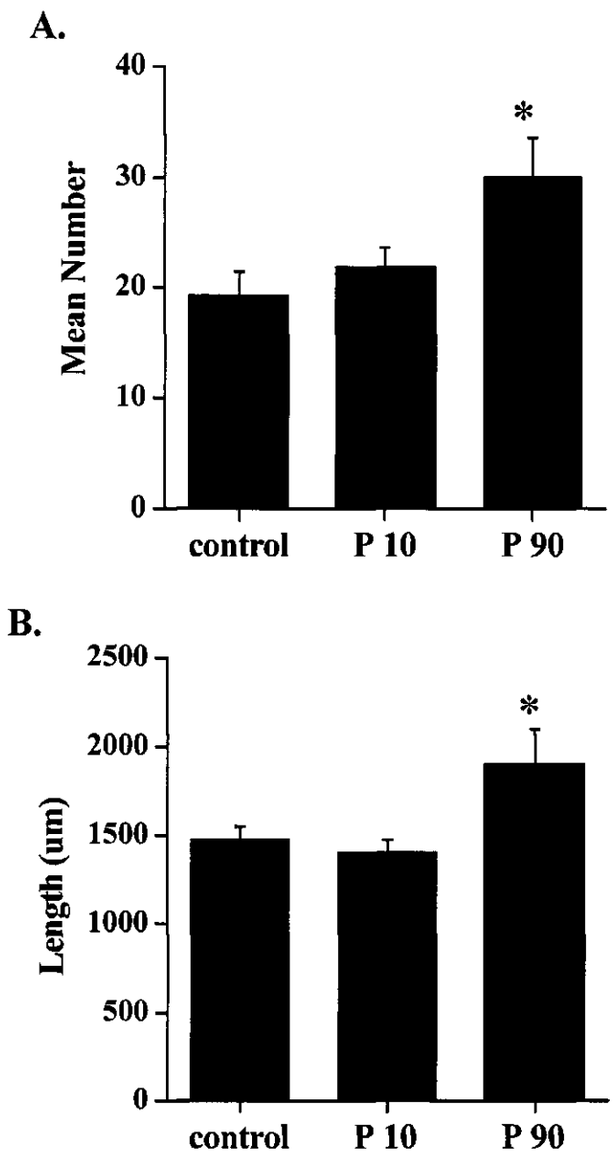

Figure 2-11. Apical Dendrites. Layer III parietal cells from the right hemisphere of both the control and hemidecorticate animals taken from Golgi-Cox stained tissue. The P 90 hemidecorticate group displayed larger and more complex cells than either other group. A. Dendritic branching. B. Dendritic length. (P 10) Neonatal hemidecorticate (P 90) Adult hemidecorticate. (* < 0.05)

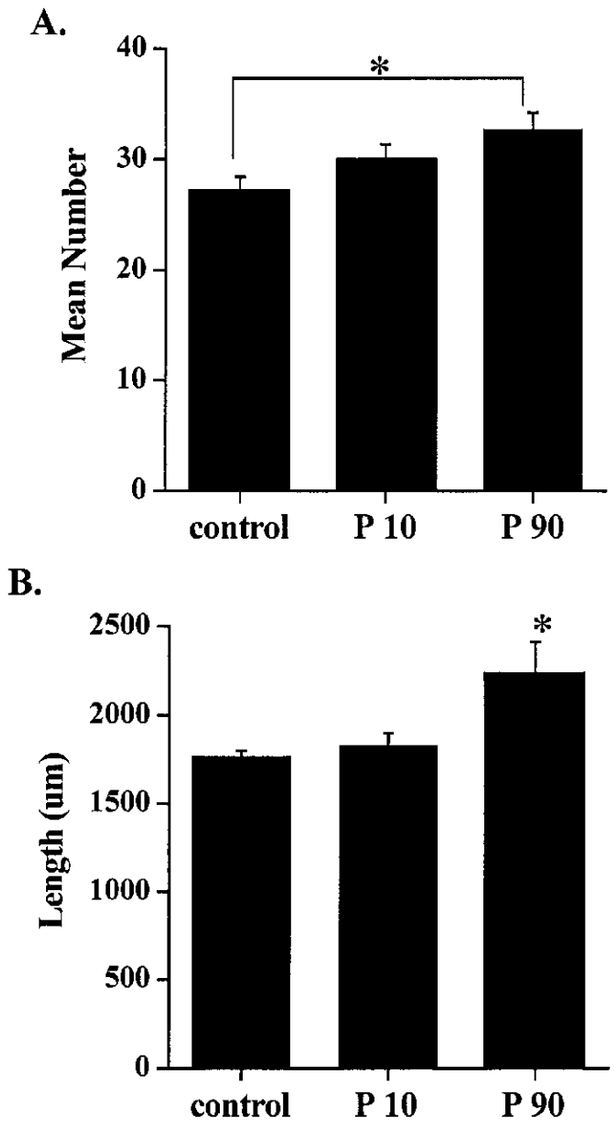

Figure 2-12. Summary of measurements of basilar fields of Layer III parietal cells from the right hemisphere of both the control and hemidecorticate animals taken from Golgi-Cox stained tissue. The P 90 hemidecorticate group displayed larger and more complex cells than either other group. A. Dendritic branching. B. Dendritic length. (P 10) Neonatal hemidecorticate (P 90) Adult hemidecorticate. (* < 0.05)

ICMS Analysis

Only females from the P 10 hemidecorticate group and controls underwent ICMS. ICMS revealed no difference in total map size between animals that had been hemidecorticated at P 10 and controls (Figure 2-13). A t-test displayed no effect of lesion [$t(6) = 0.892, p = 0.4068$]. The maps were organized differently however. There was a larger area devoted to control of the wrist in the P 10 hemidecorticate group (Figure 2-14). A t-test showed an effect of the lesion [$t(6) = 2.612, p < 0.05$].

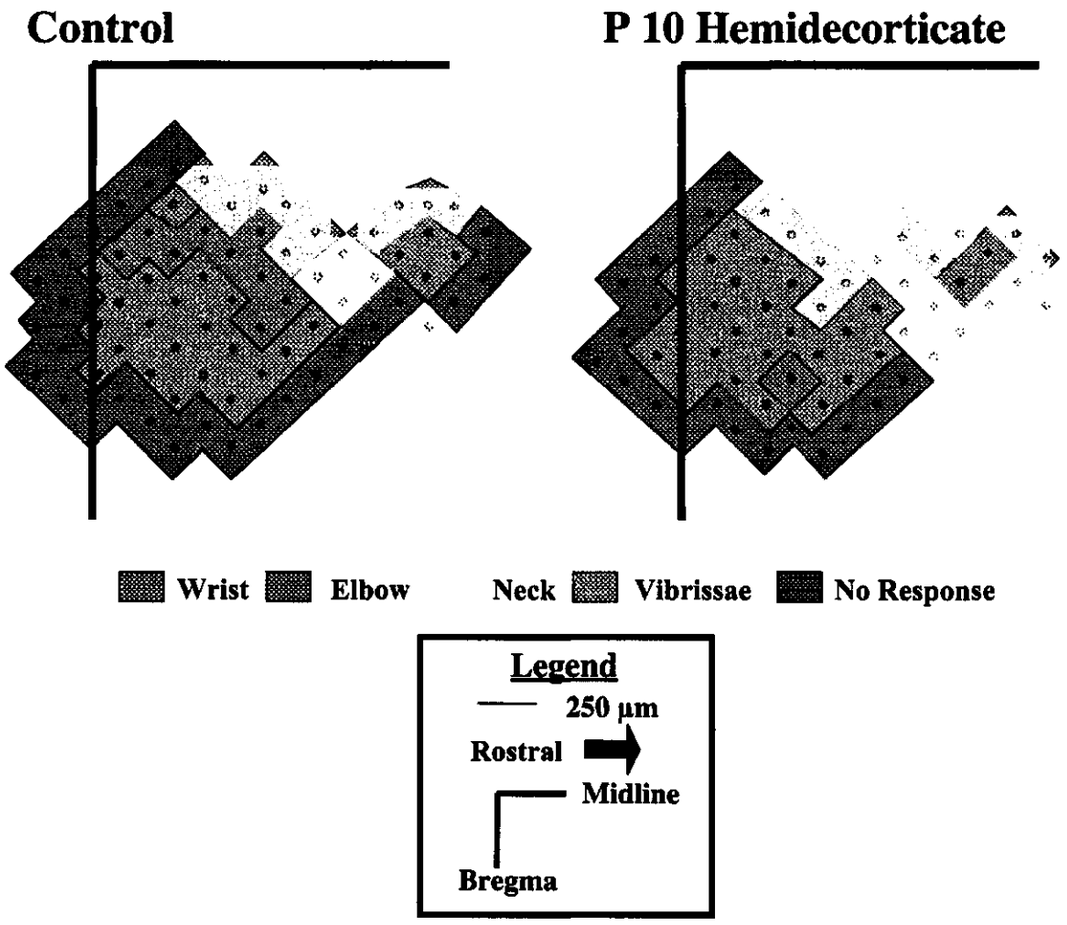

Figure 2-13. Representative maps of the caudal and rostral forelimb areas of the motor cortex. The black vertical line represents bregma. There was no difference in the overall map size but the neonatal hemidecorticates showed a large reorganization. A. control; B. P 10 hemidecorticate.

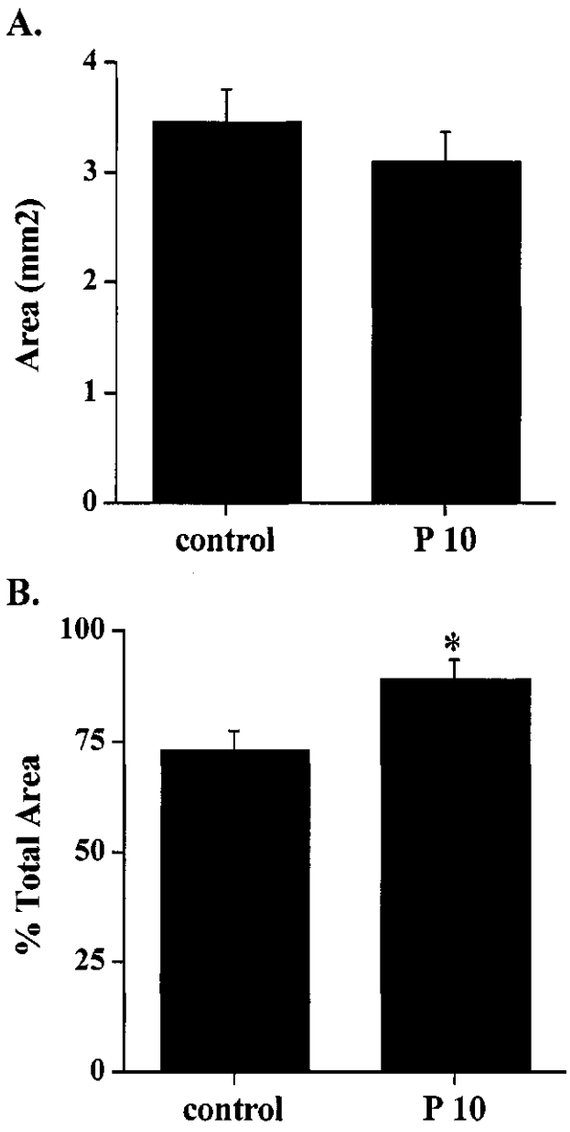

Figure 2-14. Measurements of ICMS maps of the rostral and caudal forelimb areas of the motor cortex from the P 10 hemidecorticate group and control group. A. Difference between mean map sizes. B. Comparison of wrist area in each group. (P 10) Neonatal hemidecorticate. (* < 0.05)

DISCUSSION

The objective of this experiment was to compare the forelimb capacities of rats with neonatal versus adult hemidecortications. Three main findings came out of the comparison. 1) Both groups of hemidecorticate animals were impaired when tested on the motor tasks in this experiment, but the impairments were qualitatively different in the neonatal and adult operates. 2) Hemidecortication in adults, but not P10 neonates, led to increased dendritic arborisation in the layer III pyramidal cells. 3) P10 hemidecortication altered the details of the ICMS-derived motor maps. Each of these findings will be addressed separately.

Qualitative differences in motor skill

The hemidecorticated animals were impaired at all the motor tasks used in this experiment but the adult and neonatal hemidecorticates showed different deficits. The hemidecorticate and control animals were allowed to use their preferred paw. All the brain-injured animals favoured the paw ipsilateral to their injured hemisphere. The primary difference between the P 10 and P 90 hemidecorticates was that P 10 animals showed mirror movements with their contralateral limb on three of the motor tests. That is, on the single pellet reaching, cylinder, and sunflower tasks the P 10 rats made simultaneous forepaw movements. Because of the synchronous timing of these movements and the fact that both paws were literally "mirroring" each other, these movements were dubbed mirror movements. This is one of the first reports of true mirror movements in an awake, behaving rat. Castro and Kartje (Kartje-Tillotson, O'Donoghue, Dauzvardis, & Castro, 1987) were able to elicit such movements during stimulation of the motor cortex during ICMS but they did not report them while the animals' were

awake. Curiously, in the current study, the mirror movements could be observed in freely moving animals but not under anaesthesia for the ICMS. The difference between this study and that of Castro is the lesion size. Castro only ablated frontal and motor cortex while the lesions in this study completely removed the neocortex on one hemisphere.

Mirror movements have been noted to occur in normal children until the age of ten (Rakic & Yakovlev, 1968), at which time the corpus callosum and spinal cord finish myelinating. Woods and Teuber (1978) were the first to report mirror movements in brain-injured humans, however. They noted that the earlier a child experienced a brain lesion, the more mirror movements they would produce. They hypothesized that it was a failure of the pyramidal tract to decussate. It has also been hypothesized that mirror movements may be a price for enhanced recovery (Kuhtz-Buschbeck, Sundholm, Eliasson, & Forssberg, 2000; Vulliemoz, Raineteau, & Jabaudon, 2005). Because these mirror movements are clearly demonstrated in the behavioural tasks, it is assumed that the cortico-spinal projections are responsible.

The current study demonstrated that these mirror movements have advantageous effects on behavioural tasks. The P 10 hemidecorticate animals showed a greater ease at eating the five sunflower seeds (Figure 2-6) and they were slightly less asymmetric when exploring in the cylinder task (Figure 2-5). Both of these tasks allowed for freedom of movement with both limbs. When consuming the sunflower seeds, the P 10 hemidecorticate animals were able to rotate the seeds easily but still had an impairment that was related to digit flexion that gave them difficulty in opening the seeds because it prevented the animals from getting a solid grip on the seeds and they would therefore

drop them. The deficit in the adult hemidecorticates was far worse, however, as they did not use their impaired limb for the cylinder exploration or sunflower tasks resulting in very asymmetric movements in these tasks. The animals could use their ipsilateral limb effectively, however, and were more accurate on each of their reaches in the single pellet task than even controls. One hypothesis for this improved performance is that the loss of movement from the non-reaching paw might improve the accuracy of the reaching paw.

As in past studies, unilateral cortical injuries were shown to produce deficits in the ipsilateral limb for skilled movements (e.g., Gonzalez & Kolb, 2003). This result is surprising given the morphological and electrophysiological evidence of reorganization within the intact hemisphere. These changes may provide a basis for improved motor functioning of the contralateral limb but at the price of interfering with ipsilateral limb function.

Another theory might be that both hemispheres have bilateral control of forepaw movements. By removing the cortex of one hemisphere, both contralateral and ipsilateral projections are damaged. Thus, the ipsilateral paw also displays motor deficits in tasks such as reaching.

Adult hemidecortication induced dendritic hypertrophy in the intact hemisphere.

It has been shown previously that hemidecortication at P 1 produces increased dendritic branching in the intact hemisphere (Kolb et al., 1992). The current study showed that adult hemidecortication produces similar changes but, surprisingly, P 10 hemidecortication did not. The reason for this age-related difference is not immediately apparent but there are several possibilities.

First, there may be differences in cortico-striatal or cortico-spinal projections in the day 10 animals that are not seen in the young or older operates. Kolb and colleagues (1992) have shown that cortico-fugal connections were altered in rats by P 1 hemidecortication and they presumed that these changes were related to the functional advantages. Similar findings and conclusions have also been shown in neonatally hemidecorticated kittens (Villablanca et al., 1993). Further, mirror movements have clearly been associated with alterations at the level of cortico-spinal projections (Kuhtz-Buschbeck et al., 2000; Vulliemoz et al., 2005; Woods & Teuber, 1978). Taken together these studies suggest that there may also be alterations in the cortico-fugal projections of the P10 hemidecorticates although the pattern of changes may be different than those seen in the P1 animals. In the case of the P 90 hemidecorticate animals, the cortico-spinal projections have long been established and it seems unlikely that there would be much modification after adult hemidecortication.

Second, animals with P10 frontal, complete cingulate or olfactory bulb lesions show spontaneous neuronal proliferation (e.g., Gonzalez, Whishaw, & Kolb, 2003; Kolb, Gibb, Gorny, & Whishaw, 1998). Accordingly, it is possible that there is increased neuron numbers in the striatum or cortex in the intact hemisphere. Indeed, it was noted that there appeared to be some cell proliferation along the anterior midline in the P10 hemidecorticates. Future studies should use a mitotic label such as bromodeoxyuridine to demonstrate this unequivocally.

Third, from the cortical thickness measures we know that the adult hemidecorticate group shows a decrease in thickness throughout the cortex. This most likely occurs because of the loss of projections from the contralateral cortex. What is

interesting, however, is that the cortex was not thinner in the P10 hemidecorticates. Given that the cortico-cortical connections were not established yet at P10 it is possible that these neurons developed other connections either within the cortex or to subcortical regions such as the striatum. As a result, the loss of cortex of one hemisphere did not affect the cortical thickness on the contralateral hemisphere to the same extent in the infant and adult operates.

P10 Hemidecortication produced changes in the motor map

P 10 hemidecorticates display a reorganization of the rostral and caudal forelimb areas of the motor cortex. The goal of mapping the P 10 hemidecorticate animals was to observe whether the mirror movements could be reproduced with electrical stimulation. At the electrical current amplitude used, no mirror movements were observed. The organization of the map was profoundly altered however. A greater amount of motor cortex was devoted to a representation of the wrist in both the rostral and caudal areas of the motor cortex in the neonatal hemidecorticates.

It is important to note that both the control and the hemidecorticate groups had been trained on two reaching tasks. This has previously been shown to increase the amount of area devoted to wrist control (Kleim, Barbay, & Nudo, 1998). Eighty-five percent of the caudal forelimb area was devoted to wrist (Figure 2-10). This is the first time that such a large area has been found devoted to wrist representation. It may be that the increased area would be required to control both forepaws simultaneously.

The conclusion from these data is that overall motor recovery is not better or worse after hemidecortication at different ages but simply different after injury at different ages.

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3. EVALUATING TACTILE STIMULATION AS A TREATMENT FOR NEONATAL HEMIDECORTICATION

ABSTRACT

After large neocortical lesions, such as hemidecortication, children can show significant motor and cognitive impairments. It is therefore of considerable interest to identify treatments that might enhance long-term functional outcome. The objective of the present experiment was to analyze the effectiveness of post-lesion tactile stimulation in reducing functional deficits associated with hemidecortication. Rats were given hemidecortications on postnatal day 10 (P10). Half of the group was then exposed to a daily tactile stimulation treatment for 15 minutes, three times a day for eleven days after the surgery. All groups were then tested on a number of behavioural tasks (Morris water task, skilled reaching, forelimb placing during spontaneous vertical exploration, and a sunflower seed opening task) beginning at P 120. The brains of the male animals were prepared for Golgi-Cox staining and subsequent analysis of dendritic arborisation and spine density. There were two main findings in this experiment: 1) Tactile stimulation improved cognitive and some motor performance after P 10 hemidecortication; and, 2) Tactile stimulation does not alter cortical thickness, nor dendritic length or arborisation in Layer III parietal pyramidal cells.

INTRODUCTION

Perinatal neocortical injury can devastate motor and cognitive function in both humans and laboratory animals. The severity of the behavioural deficits varies with precise age at injury as well as the nature and location of the insult (Kolb, 1995; Kolb, 2001). The severe effects of large neocortical injuries are problematic clinically, especially for children with extensive neocortical resections for neurological disorders such as Rasmussen's encephalitis (Rasmussen, 1983). This encephalitic disorder results in severe seizures that are generally unresponsive to drug treatments but can be alleviated with the removal of affected areas of the brain in children. Unfortunately, it is often necessary to perform extensive resections that include the complete neocortex of one hemisphere of the brain or even the entire hemisphere itself. Although there are isolated reports of remarkable recovery in children with such surgeries (Smith, 1966), This is not common and most children with such large removals have severe cognitive and motor deficits with an average IQ of about 85 (Vargha-Khadem & Polkey, 1992). It is therefore of considerable interest to identify treatments that might enhance long-term functional outcome.

Postnatal day ten (P 10) hemidecortication is a model of large neocortical lesion. When rats receive large neocortical insult at this stage of development robust motor and cognitive deficits can be observed (Kolb & Tomie, 1988). Although there are changes in the corticospinal connections of the intact hemisphere (e.g., (Whishaw & Kolb, 1988), there is surprisingly little spontaneous change in cell morphology in the neurons of the intact hemisphere (e.g., see previous chapter). Given that previous studies have shown significant benefits of a variety of treatments for animals with focal perinatal injuries

(e.g., Kolb, 1995), and the absence of spontaneous changes in the intact hemisphere, we hypothesized that it might be possible to sitmulate significant change in the intact hemisphere and that this, in turn, would facilitate functional improvement.

One treatment that has been shown to improve cognitive and motor recovery after focal brain injury is tactile stimulation of the skin. This involves petting the skin of the subject with a soft brush for 15 minutes three times a day. This method has been shown to prevent hippocampal damage in rats that are submitted to neonatal hypoxia-ischemia (Rodrigues et al., 2004). It also increases growth factors such as Epidermal Growth Factor (EGF) (Donnelly, Hoath, & Pickens, 1992) and basic Fibroblast Growth Factor (FGF-2) in the skin and brain tissue of rats (Gibb, 2001). Tactile stimulation also appears to have a metabolic effect as it increases serum lactate by 207% in the brains of newborn rats (Alasmi, Pickens, & Hoath, 1997). It was not clear, however, if this treatment would improve the functional outcome after large neocortical injury.

The objective of this study was to use tactile stimulation as a treatment to improve motor and cognitive function after perinatal hemidecortication. The animals were hemidecorticated at P 10 and submitted to the tactile stimulation treatment for eleven days afterward. The animals were then tested on several behavioural tasks at the age of four months. Their brains were then prepared for Golgi-Cox staining and subsequent analysis of dendritic arborisation.

METHODS

Subjects

Subjects were 35 male and 29 female Long-Evans hooded rats. All animals were raised in the University of Lethbridge vivarium and were housed in groups of 4-6 in hanging plastic tubs. The colony room was controlled on a 12 hr light: 12 hr dark cycle (lights on 07.30-19.30 hr) and the temperature maintained at 22°C. Experiments were conducted according to standards set by Canadian Council on Animal Care and approved by the University of Lethbridge animal care committee.

Surgical Procedures

At ten days of age (postnatal day 10 or P 10) all rats were anesthetized by cooling them in a Thermatron cooling chamber until their body temperatures were in the range of 18-20°C. Surgery was performed using suction by pipette with the aid of a surgical microscope (Kolb, Sutherland, & Whishaw, 1983). Rats received either a complete left hemisphere decortication or a sham incision. Pups were warmed up by hand and then placed on a warm pad until all of the animals were moving. They were then returned to the dam.

Treatment Procedures

Half of the lesion group and half of the sham group received 15 minutes of tactile stimulation 3 times a day until weaning at postnatal day 21. The tactile stimulation was performed with a standard size Clinique blush brush.

Food restriction

Subjects were kept on a restricted food regime. Each animal received 20g of food per day after the testing session was complete. Their body weight was maintained at ≈ 90-95% until the completion of the behavioural testing.

Morris Water Task

The maze consists of a circular pool (diameter 1.5 m, height 45 cm), the inside of which was painted white and filled with water at about 24°C mixed with instant powdered milk. A clear Plexiglas platform (11cm x12 cm) was hidden inside the pool (Kolb et al., 1983). (See illustration, Figure 1-5)

One trial consisted of placing the rat into the pool, facing the wall of the pool, at one of four starting locations. Four trials per day were carried out, each trial beginning at a different starting location. Each day the order of the starting locations was altered.

A computer tracking system measured the distance swum by the rat from the starting location until it reached the platform.

Tray reaching

The animals were placed in a test cage (10x18x10 cm high) with floor and fronts constructed of 2 mm bars, 9 mm apart edge to edge. A 4 cm wide and 1 cm deep tray, containing chicken feed pellets, was mounted in the front of each box (Figure 1-6).

The rats were food deprived to 90% of their normal body weight for testing. The rats were required to extend a forelimb through the gap in the bars, grasp and retract the food and eat it. Animals were tested every day for three weeks in the tray reaching apparatus. They were allowed to reach with either paw. At the end of each test week,

rats were filmed for 5 mins and the tapes were scored for numbers of attempted and successful reaches. The percent success was calculated as follows:

(Successful reaches/ (successful reaches + attempts)) x 100%

This was calculated for each week and then averaged.

Single Pellet reaching

The single pellet reaching boxes were made out of clear Plexiglas with the dimensions 45cm deep x 14cm wide x 35cm high. In the centre of the front wall was a 1 cm wide slit which extended from 2 cm above the floor to 15 cm above (see Figure 1-7). In front on the slit on the outside of the box there was a 2 cm wide shelf. The shelf was placed 3 cm above the floor. Two indentations were made on the shelf 2 cm from the inside of the wall. The indentations were centred to the slit so the rats could easily reach them. Food pellets (45 mg Rodent Chow food pellets, Bioserve) were placed in the indentation opposite to the rats' preferred reaching paw.

The animals were trained as adults for 2 weeks and then tested for nine days on the single pellet reaching task. They were presented with 20 pellets in each testing session. A successful reach was defined as the pellet being retrieved from the platform and eaten by the animal. At times the rat would reach and miss the pellet but repeat the reaching movement until it finally retrieved the pellet. Each reaching movement was counted as an attempt. The performance of each animal was analyzed in two ways. First, the number of successful reaches out of twenty trials was analyzed. Second, the accuracy of each reach was analysed. This was calculated as follows:

Number of attempts/ # of pellet successfully retrieved

An attempt was classified as the motion of reaching for the pellet. A score of 1 is perfect, as it would take one attempt to retrieve one pellet.

Forepaw Asymmetry

Forelimb use during spontaneous exploration was examined by placing rats in a transparent cylinder 20 cm in diameter and 30 cm in height (Schallert, Kozlowski, Humm, & Cocke, 1997). A mirror was placed beneath the cylinder to allow the activity of the rat to be videotaped. For the video analysis, each forepaw contact with the cylinder wall was counted (Figure 1-8). The asymmetry score [i.e. contralateral forelimb/ (contralateral + ipsilateral)] of forepaw use was calculated for both the first touch of any bout of exploration and the total amount of touches for the testing period. Animals were individually placed in the cylinder for a single, three minute testing session.

Sunflower Seed Task

Five sunflowers seeds were placed in the corner of a clear Plexiglas box (50 x 50 x 50cm). The rat was allowed to explore the box until it discovered the seeds. Rats began by manipulating the seeds into their preferred position before removing the shell (Figure 1-8). The animal would then eat all five seeds in succession. The total amount of time which the animal spent manipulating, opening, and consuming the seeds was recorded. Animals were trained for one day and tested on the second and third day.

Anatomical Procedures

The animals were separated by sex for the anatomical studies. Only male brains were stained with Golgi-Cox and only female brains were mapped with ICMS.

Male animals were given an overdose of sodium pentobarbital and perfused intracardially with 0.9% saline. The brains were removed and weighed before being

submerged in ~20mL of Golgi-Cox solution. The brains were left in this condition for 14 days and then placed in a 30% sucrose solution for two days. The brains were cut on a vibratome at 200μm and developed using the procedure described by Gibb & Kolb (1998). Layer III pyramidal cells were traced in Zilles' parietal area 1 using camera lucida at 250X magnification. Dendritic length and branching were measured on these drawings. Branch order analysis was done according to Coleman & Riesen (1968). Branch length also was measured indirectly by Sholl analysis (1956).

Cortical Thickness

Cortical thickness was measured on the Golgi-Cox stained brains. Only males were used for this analysis. The dimensions were taken on a Zeiss 2 POL projector set at a magnification of 10x. Measurements were taken at three different points at each of five planes corresponding to Zilles' levels +2.2, -0.3, -2.3, -4.8, and –6.3 relative to bregma, (Stewart & Kolb, 1988). The planes were chosen for their correspondence to the anterior tip of the caudate-putamen, the middle of the anterior commisure, the rostral tip of Ammon's horn, the posterior commisure, and the posterior tip of the hippocampus. The multiple measurements from each plane were averaged and then all five planes were averaged again.

Statistical Analysis

Analyses of variance (ANOVA) were used for all measurements and Fisher's LSDs ($p< 0.05$ or less) were used for *post hoc* evaluations. Because no sex differences could be measured the data were collapsed across this factor.

BEHAVIOURAL RESULTS

Morris Water Task

Overall, the hemidecorticated animals were severely impaired in the Morris water task [F (1,61) = 19.812, $p < 0.0001$] but showed significant benefits from the tactile stimulation. There was a main effect of treatment [F (1,61) = 4.120, $p< 0.05$] but no Lesion x Treatment interaction [F (1,61) = 2.145, $p = 0.1482$].

Figure 3-1A shows the average distance that each group swam for each of the testing days. A two-way repeated measures ANOVA demonstrated a main effect of day [F (4,244) = 54.248, $p<0.0001$] showing that all groups improved to some degree. There was also a significant Day x Lesion interaction [F(4,244) = 4.41, $p < 0.05$].

It is apparent from Figure 3-1A that although the tactile stimulation had no effect on the control animals, there did appear to be a benefit for the hemidecorticates but this did not show up in the three way interaction. The data therefore were reanalyzed by summing across all trial blocks (Figure 3-1 B). A Fisher's LSD test showed that the injured animals that received tactile stimulation were able to perform the task significantly better than their untreated counterpart ($p = 0.01$).

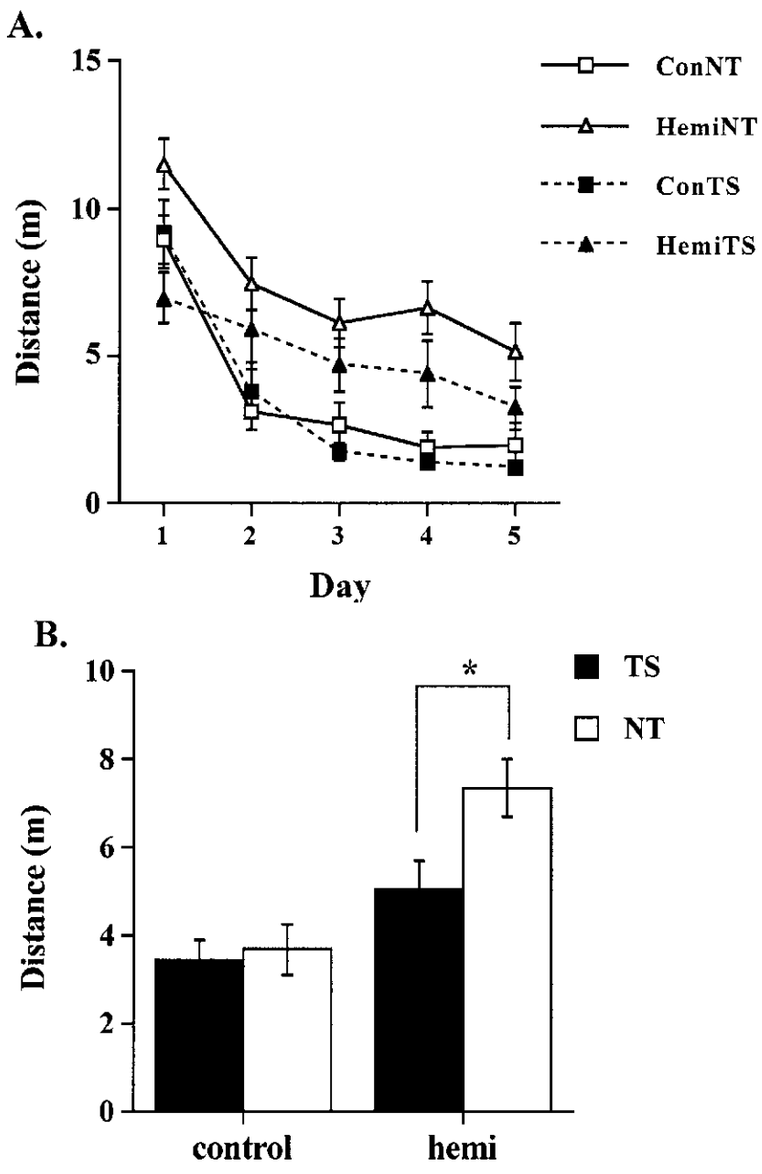

Figure 3-1. Morris water task. The hemidecorticated animals were impaired at this task. The tactile stimulation treatment improved the performance of the injured animals significantly. A. Distance traveled to reach the platform in the Morris water task each day. B. Overall average distance over all five days. Hemi; P 10 hemidecorticate, TS; tactile stimulation, NT; no treatment. (* < 0.05)

Tray Reaching

The animals were allowed to use whichever paw they preferred in the tray reaching task (Figure 3-2). As might be expected, the hemidecorticate all rats used their ipsilateral forepaw to reach for the food. Nonetheless, as reported previously (Whishaw & Kolb, 1988), the hemidecorticate groups showed an overall impairment [$F(1,62) = 11.267, p < 0.05$]. Tactile stimulation did not improve reaching performance [$F(1,62) = 0.901, p = 0.3463$]. There was also no Lesion x Treatment interaction [$F(1,62) = 0.952, p = 0.3331$]. Inspection of Figure 3-2 shows that the tactile stimulation appeared to provide some benefit for the hemidecorticates and this was born out by planned *post hoc* tests. A Fisher's LSD showed that the non treated hemidecorticate group was significantly impaired in comparison with both the treated ($p < 0.01$) and the non-treated controls ($p < 0.0001$). The treated hemidecorticate group did not show significant impairment when compared with either control group however, confirming the impression that there was some benefit.

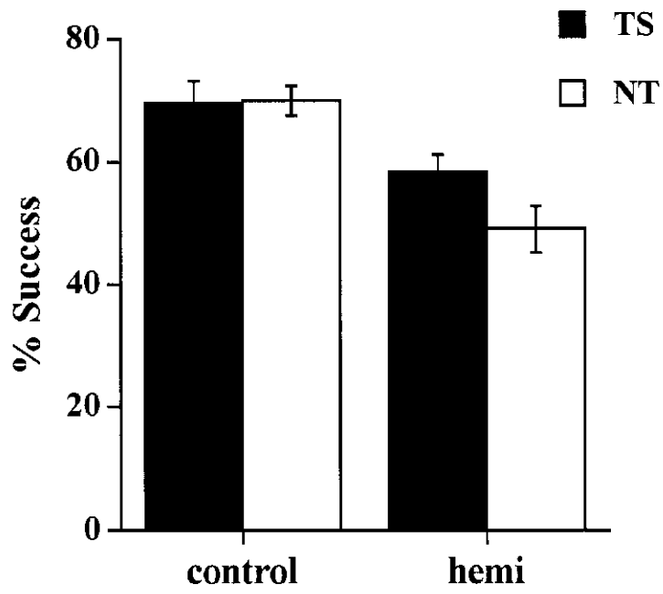

Figure 3-2. Tray Reaching Success. The hemidecorticate groups were both impaired at this task. The treatment had no effect on either the control or injured groups. The graph shows the average success over three weeks in the tray reaching task. Hemi; P 10 hemidecorticate, TS; tactile stimulation, NT; no treatment.

Single Pellet Reaching Task

Again the hemidecorticate animals used their ipsilateral paw to reach for a food pellet in this task. These animals displayed the mirror movements that were discussed in the previous chapter (Figure 2-3). These movements involved the animals using both paws in synchrony. That is, the contralateral (impaired) paw would actually mirror the motions of the ipsilateral paw. The treatment did not affect either the lesion or control group in their ability to retrieve a pellet, nor did it alter the mirror movements.

There were a total of 20 pellets (or trials) for each animal. The average number of pellets retrieved was not affected by the treatment [$F(1,35) = 1.270, p = 0.2674$] (Figure 3-3 A). The hemidecorticate groups were both significantly impaired at successfully retrieving the pellets [$F(1,35) = 17.177, p < 0.01$]. There was no Lesion x Treatment interaction [$F(1,35) = 0.009, p = 0.9250$] when measuring the total number of pellets retrieved.

The reaching accuracy was also measured in this task (Figure 3-3B). A perfect reach requires one reach/pellet successfully retrieved. There was no main effect of lesion [$F(1,35) = 2.086, p = 0.1576$] or treatment [$F(1,35) = 2.631, p = 0.1138$] for the accuracy of retrieving a pellet. There was also no Lesion x Treatment interaction [$F(1,35) = 0.203, p = 0.6550$].

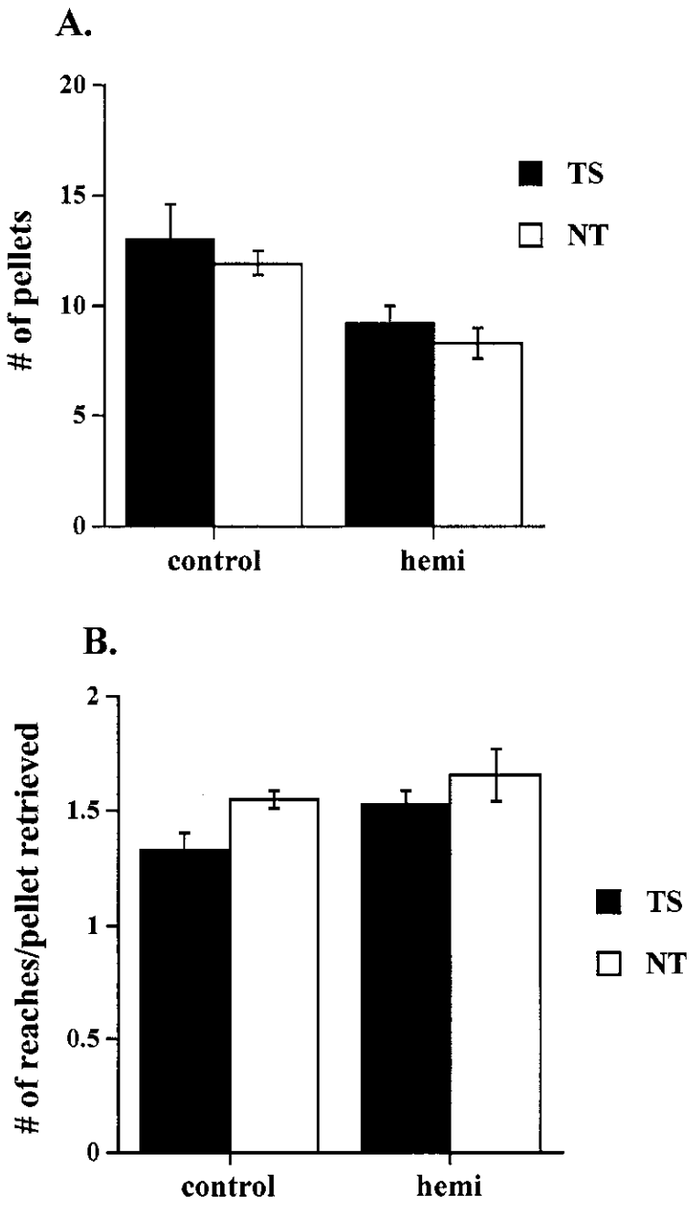

Figure 3-3. Single pellet reaching task. The P 10 hemidecorticate animals were significantly impaired at successfully retrieving pellets in this task. The treatment had no effect on either group. A. Total number of pellets that rats successfully retrieved. B. Accuracy measured by calculating the number of reaches for each successful retrieval. Hemi; P 10 hemidecorticate, TS; tactile stimulation, NT; no treatment.

Forepaw Asymmetry

The control animals used both forepaws equally while exploring a novel, cylindrical environment (Figure 3-4). The hemidecorticated animals were significantly more asymmetrical than the control group [F (1,61) = 24.612, $p < 0.0001$] and were more reliant on their ipsilateral-to-lesion paw. The tactile stimulation did not affect either group of animals [F (1,61) = 0.286, $p = 0.5949$]. The two-way ANOVA also showed no Lesion x Treatment interaction [F (1,61) = 1.616, $p = 0.1827$].

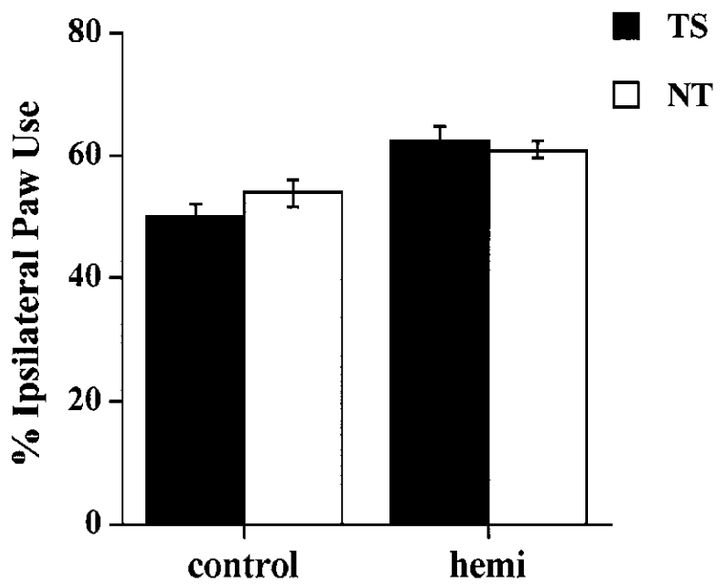

Figure 3-4. Forepaw asymmetry. Hemidecorticated rats relied on their ipsilateral-to-lesion paw more often while exploring a novel environment. Hemi; P 10 hemidecorticate, TS; tactile stimulation, NT; no treatment.

Sunflower Seed Consumption Task

In the sunflower seed consumption task, time was measured for an animal to consume five sunflower seeds (Figure 3-5). The hemidecorticate animals were severely impaired at this bimanual task [$F(1,58) = 13.261, p < 0.01$]. Tactile stimulation significantly improved the overall performance of this task [$F(1,58) = 5.357, p < 0.05$]. The Lesion x Treatment interaction was not significant however [$F(1,58) = 0.285, p = 0.5956$]. The treated hemidecorticate group was significantly better than their non-treated cohorts ($p < 0.05$). The treated hemidecorticate group was not different than the non-treated controls ($p = 0.3568$) but was impaired when compared with the treated controls ($p = 0.05$).

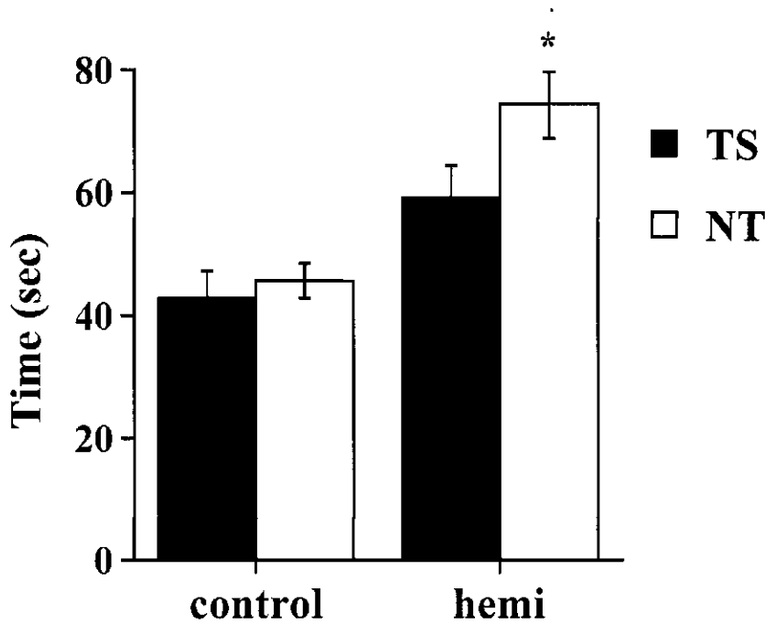

Figure 3-5. Sunflower Seed Consumption Task. Time measured to consume five sunflower seeds. The hemidecorticate animals that received the tactile stimulation treatment were no different than controls at this task. The non-treated hemidecorticates were impaired when compared to all other groups. Hemi; P 10 hemidecorticate, TS; tactile stimulation, NT; no treatment. (* < 0.05)

ANATOMICAL RESULTS

The brains were removed after intracardial perfusion. The brains from male rats were placed in Golgi-Cox solution for a period of two weeks (Figure 3-6). There was some variation in the lesion sizes of the hemidecorticate animals. The olfactory bulbs on some of the injured animals were slightly misshapen on the ipsilateral side.

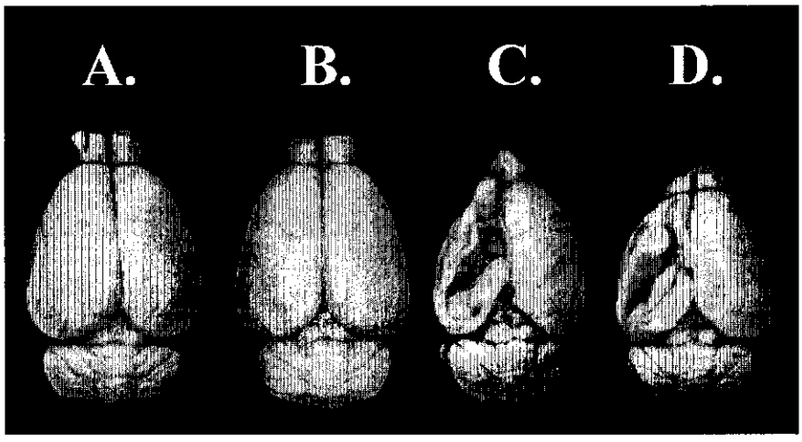

Figure 3-6. Representative brains from the different groups of animals. A; control with tactile stimulation, B; untreated control, C; P 10 hemidecorticate with tactile stimulation, D; untreated P 10 hemidecorticate.

MaleBrain Weight

The male brains were compared for weight. There was an obvious effect of hemidecortication [$F(1,29)= 44.577, p < 0.0001$]. There was not, however, any effect of the tactile stimulation [$F(1,29)= 0.241, p= 0.6271$]. There was also no Lesion x Treatment interaction [$F(1,29)= 0.477, p= 0.4954$].

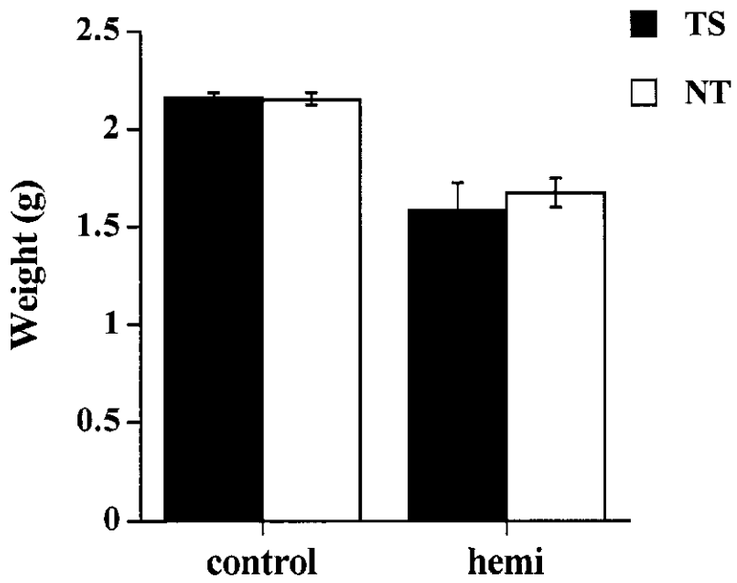

Figure 3-7. Mean brain weight of male rats. There was an effect of hemidecorticate on the weight of the brains but no effect of the tactile stimulation. Hemi; P 10 hemidecorticate, TS; tactile stimulation, NT; no treatment.

Cortical Thickness

Neocortical thickness was measured in the intact hemispheres of the male brains. There was no effect of the P 10 hemidecortication on mean cortical thickness [$F(1,12) = 0.647, p = 0.4367$], nor was there a main effect of the tactile stimulation [$F(1,12) = 0.705, p = 0.3272$]. There was also no Lesion x Treatment interaction [$F(1,12) = 1.044, p = 0.3272$].

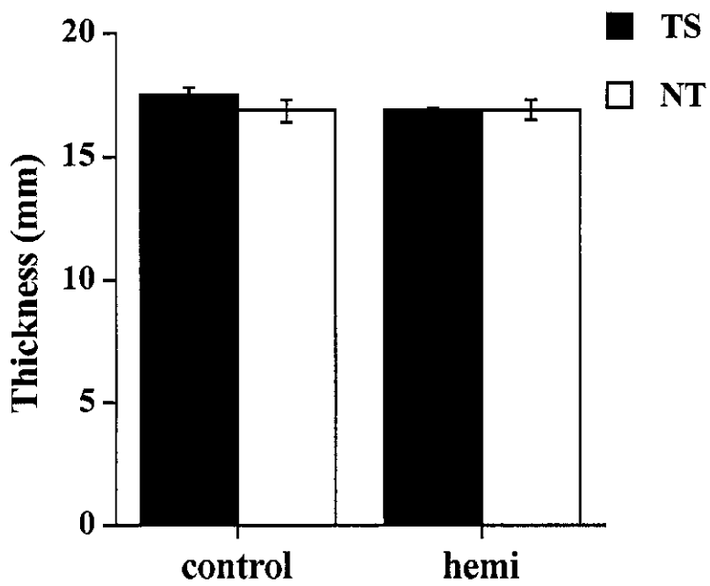

Figure 3-8. Cortical thickness measurements. Thickness of the neocortex was measured throughout the brain. The measurement recorded reflects the thickness at 20x magnification. There was no effect of hemidecortication or treatment. Hemi; P 10 hemidecorticate, TS; tactile stimulation, NT; no treatment.

Golgi-Cox Analysis

The overall finding was that whereas hemidecortication increased the arborisation of the basilar dendrites of layer III pyramidal cells, there was no effect of treatment. Cells were drawn in the hemisphere contralateral to injury. Analysis of the apical branching complexity (Figure 3-9) showed was no effect of the lesion [F (1,13) = 2.1650, $p = 0.1650$] or treatment [F (1,13) = 0.389, $p = 0.5434$]. There was also no Lesion x Treatment interaction [F (1,13) = 0.154, $p = 0.7008$] for the apical branch order analysis. Analysis of apical dendritic length of the apical tree also showed no change by either the lesion [F (1,13) = 4.445E-5, $p = 0.9948$] or treatment [F (1,13) = 0.116, $p = 0.7393$], and there was no Lesion x Treatment interaction [F (1,13) = 0.859, $p = 0.3709$].

Analysis of the basilar branching complexity (Figure 3-10) also showed a significant main effect of lesion [F (1,13) = 5.401, $p = 0.0370$], but there was no main effect of treatment [F (1,13) = 0.350, $p = 0.5645$] or Lesion x Treatment interaction [F (1,13) = 0.015, $p = 0.9034$], however. Similarly, the dendritic length of the basilar tree was not affected by the lesions [F (1,13) = 0.004, $p = 0.9501$] or the treatment [F (1,13) = 0.455, $p = 0.5119$] and there was no Lesion x Treatment interaction [F (1,13) = 0.060, $p = 0.8103$].

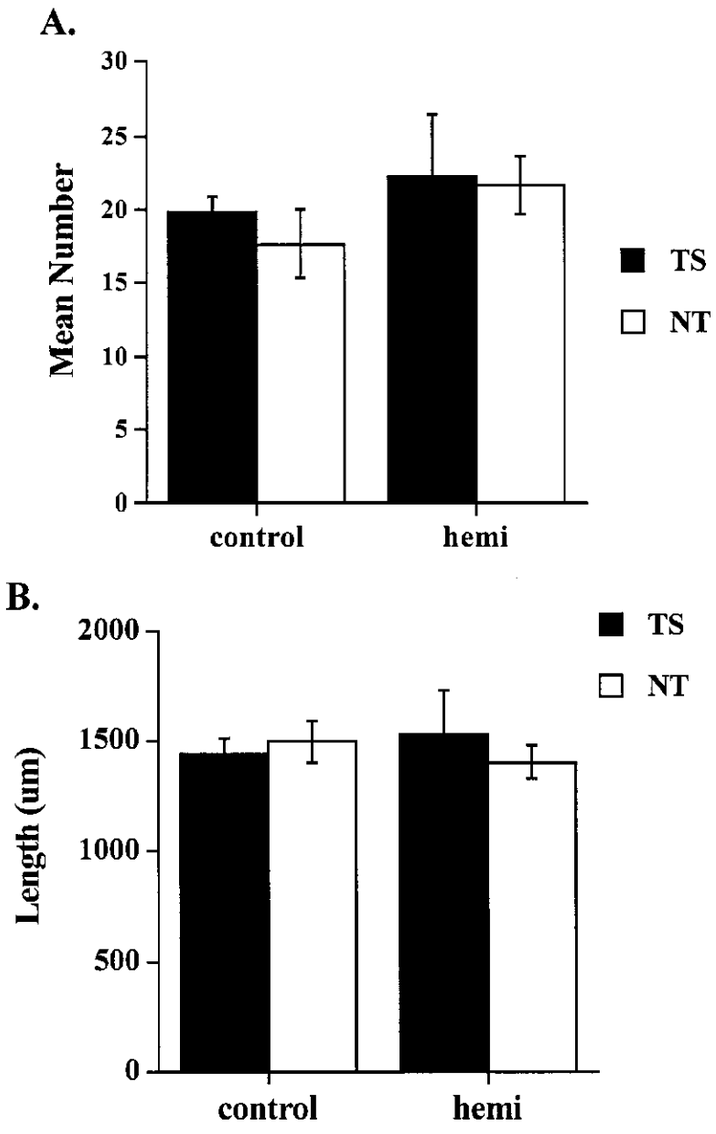

Figure 3-9. Apical branch analysis. Layer III parietal cells were drawn in the right hemisphere (contralateral to injury) of both the control and injured animals. There was no effect of hemidecortication on either the length or complexity of the apical branches. A. Branch order analysis. B. Dendritic length.

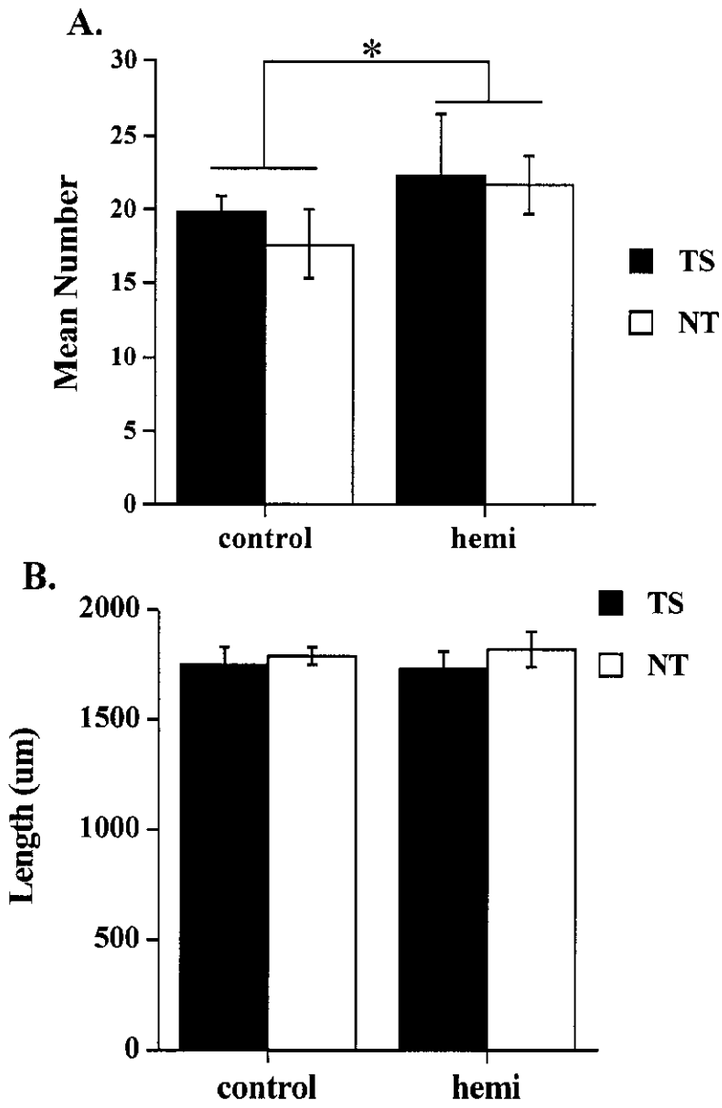

Figure 3-9. Basilar branch analysis. Layer III parietal cells were drawn in the right (contralateral to lesion) hemisphere of both the control and hemidecorticate animals. There was an effect of hemidecortication on the complexity of the basilar branches but no effect of treatment on either complexity or dendritic length. A. Branch order analysis. B. Dendritic length. (* < 0.05)

DISCUSSION

Tactile stimulation improved functional outcome on the tray reaching tack, sunflower seed consumption task and the Morris water task. There was no effect of the stimulation on either the single pellet task or the forepaw asymmetry task. One explanation of the selective motor benefits could be related to the nature of the tasks and the mirror movements observed in the hemidecorticates. That is, it is possible that the mirror movements were beneficial and that the tactile stimulation somehow enhanced this benefit. Mirror movements are not beneficial in the single pellet task because the apparatus constrains the movements (Figure 1-7). The forepaw asymmetry task is unlike the other motor tasks as there is no particular benefit in using one versus two limbs to explore and the asymmetry in the lesion animals is rather small to begin with so we might no predict any stimulation-related effect on this task.

Two explanations for the tactile stimulation effects are considered: 1) changes in cerebral organization; and 2) changes in stress reactivity.

Changes in cerebral organization

One major effect of neonatal hemidecortication is a reorganization of the cortico-spinal tract (e.g., Whishaw & Kolb, 1988). It is possible that the tactile stimulation affected this reorganization, although that would be difficult to quantify. There was an increase in the arborisation of the basilar dendrites of layer III pyramidal neurons in sensorimotor cortex in the intact hemisphere, which could reflect a reorganization of the sensorimotor networks. Previously (Chapter 2) it was shown that P10 hemidecortication altered the organisation of the ICMS-identified motor maps and it is possible that the tactile stimulation further altered these maps.

Although tactile-stimulation-induced changes in motor organization might account for the benefits in the motor tasks, it is not immediately obvious how such changes would benefit spatial learning. It seems more likely that either the tactile stimulation altered the organization of the prefrontal cortex or hippocampus or that the benefits in spatial learning were mediated by some other mechanism (see below).

A question that arises relates to the mechanism whereby tactile stimulation might affect cerebral organization. It has been shown previously that tactile stimulation increases the expression of a variety of proteins both in skin and brain (Gibb, 2004) and these may provide a clue. For example, Gibb showed that there was an increase in the expression of FGF-2 and FGF-2 receptor, as well as in glucocorticoid receptor in brain. Exogenous administration of FGF-2 stimulates functional recovery from focal motor cortex lesions (Monfils et al., 2005) and alters the organization of the motor maps. It is therefore possible that the tactile stimulation acts by increasing endogenous FGF-2 and this, in turn, alters cerebral organization and function.

Alteration in stress response

The behavioural improvements may also be due to a decrease in stress reactivity. Previous studies have demonstrated that neonatal handling reduced stress and age-related impairments associated with the hippocampus (Meaney, Aitken, van Berkel, Bhatnagar, & Sapolsky, 1988). This group showed that handling rats decreased levels of glucocorticoids (a stress hormone) at all ages of life. Stress can interfere with both cognitive and motor tasks. It may be that the improvement noted in the Morris water task (Figure 3-1) was due to the decreased levels of glucocorticoids. Future studies will need

to test for levels of these hormones in the system of animals that receive the tactile stimulation treatment.

Tactile stimulation does not alter cell morphology in Layer III parietal cells.

The layer III pyramidal cells of the parietal cortex have previously shown robust changes due to treatment or injury (Gonzalez, Gibb, & Kolb, 2002; Williams, Brown, & Vorhees, 2004). The current experiment showed that after P 10 hemidecortication, the basilar dendrites of Layer III cells become significantly more complex (Figure 3-10), a result that was a nonsignificant trend in Chapter 2. Previous studies have demonstrated that animals that received hemidecortication at postnatal day 1 (P 1) also show a greater complexity in the basilar tree of parietal layer III cells (Kolb, Gibb, & van der Kooy, 1992). What is interesting, however, is that P 1 hemidecorticates display an even greater behavioural sparing than was noted in the P 10 animals.

This behavioural-anatomical correlation (earlier has bigger effects) is rather different than the effects of focal lesions (e.g., Kolb, 1995) in which earlier had much smaller effects. It thus appears that there is some fundamental difference between extensive and focal injury during development. One possibility is that in the focal injury there is both an intact hemisphere and a damaged hemisphere. If we hypothesize that the brain will initiate reparative processes in the damaged hemisphere of the focally-injured brains as well as initiating some other form of "regenerative" processes in the intact hemisphere in both the focally- and extensively-injured brains, then we could have a mechanism to account for the apparent differences in the two models. Thus, we can speculate that the reparative processes in the focally-injured hemisphere are ineffective, and possibly damaging, in the first week of life whereas the regenerative processes in the

intact hemisphere are beneficial. Consider, for example, that focal lesions in the first week have profound effects on cortical connectivity and lead to generalized dendritic atrophy (Kolb, Gibb & van der Kooy, 1994) whereas lesions in the second week do not reorganize cortical connectivity and lead to dendritic hypertrophy and increased spine density (Kolb & Gibb, 1992; Kolb, Gibb & van der Kooy, 1994). In contrast, there is greater cerebral reorganization and dendritic hypertrophy after earlier hemidecortications (e.g.,Kolb et al., 1992). It remains to be seen whether there is a difference in the effects of postinjury treatments in the early versus later hemidecortications.

It may be that there is a limit to how much improvement can be accounted for by these dendritic changes. Kolb (1995) has shown that the resources available for plasticity are different at P 1 than at P 10. The behavioural improvement seen in the P 1 rat must, therefore, be due to more than just the dendritic branching.

There does not appear to be any anatomical changes due to the treatment itself. Tactile stimulation may affect dendritic spines but that was not measured in this experiment.

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4. EVALUATING BASIC FIBROBLAST GROWTH FACTOR (FGF-2) AS A TREATMENT AFTER NEONATAL HEMIDECORTICATION

ABSTRACT

Fibroblast Growth Factor –2 (FGF-2) is an important neurotropic factor that is involved in both development and brain repair after injury. The goal of this project was to analyze the functional outcome of animals that received FGF-2 injections after postnatal day ten (P 10) hemidecortication. After large neocortical lesions, such as hemidecortication, children can show significant motor and cognitive impairments. It thus is of considerable interest to identify treatments that might enhance long-term functional outcome. The objective of the present experiment was to analyze the effectiveness of exogenous treatment with FGF-2 on recovery from neonatal hemidecorticaion. Rats were given hemidecortications on P 10 and half of the subjects were then given subcutaneous injections of FGF-2 for one-week. All groups were then tested on a number of behavioural tasks (Morris water task, skilled reaching, forelimb placing during spontaneous vertical exploration, and a sunflower seed opening task) beginning at P 120. The brains of the male animals were prepared for Golgi-Cox staining and subsequent analysis of dendritic arborisation and spine density. The main finding of this experiment was FGF-2 was not beneficial and may have been detrimental to functional recovery.

INTRODUCTION

Basic Fibroblast Growth Factor (FGF-2) is one of nine members in the FGF family. This growth factor is involved in cellular processes such as the mitogenic response of endothelial cells (Bieger & Unsicker, 1996). Different isoforms of FGF-2 can be found in neurons and are important for normal brain development. For example, FGF-2 is important for proper cell pruning during development (Abe & Saito, 2001) and a loss of FGF-2 is thought to lead to abnormal cytoarchitecture (Ortega, Ittmann, Tsang, Ehrlich, & Basilico, 1998). Furthermore, blocking FGF-2 impairs functional recovery after motor cortex lesions in adult rats (Rowntree & Kolb, 1997).

One case in which FGF-2 might be beneficial is after brain injury during development as this factor has been associated with improved recovery after cortical lesions (Gibb, 2004). For example, it has been shown to support the survival of cortical projections (Catapano, Arnold, Perez, & Macklis, 2001), enhance axonal sprouting (Ramirez et al., 1999), and to stimulate neonatal and adult brain neurogenesis (Wagner, Black, & DiCicco-Bloom, 1999).

Previous studies by Gibb (2004) have shown that treatments such as tactile stimulation increase FGF-2 in both the skin and brain tissue of the rat. Tactile stimulation promotes some improvement after P 10 hemidecortication, as seen in the previous chapter of this thesis. It was therefore of interest to explore whether FGF-2 was the component of tactile stimulation that was responsible.

Other studies have demonstrated improved behavioural outcome from FGF-2 following postnatal day three (P 3) bilateral medial frontal or posterior parietal lesions (Gibb, 2004; Hastings, 2003) and postnatal day ten (P 10) motor cortex lesions (Monfils

et al., 2005). Monfils' findings are especially interesting because the animals showed a filling in of the lesion cavity with newly generated cells, which was thought to be at least partly responsible for a behavioural recovery. These experiments were all done with bilateral P 10 focal lesions, however. Thus, it was unknown how the treatment would work on large, unilateral lesions.

The objective of the present study was to analyse the motor and cognitive function of hemidecorticate rats that received FGF-2 injections. Because one of the goals of this experiment was to compare with the rats that received tactile stimulation the age of lesion was kept constant. Therefore, rats were hemidecorticated at P 10 and given FGF-2 injections using the same procedure as Monfils *et al.*, 2005. The animals were then tested on a number of behavioural tasks at P 120. A broad battery of tests was performed in order to observe the behavioural performance of all animals. Following behavioural testing, the rats were perfused, the brains prepared for Golgi-Cox staining and subsequent analysis of dendritic arborisation were performed.

METHODS

Subjects

Subjects were 30 male and 33 female Long-Evans hooded rats. All animals were raised in the University of Lethbridge vivarium and were housed in groups of 5 in hanging plastic tubs. The colony room was controlled on a 12hr light: 12 hr dark cycle (lights on 07.30-19.30h) and the temperature maintained at 22°C. Experiments were conducted according to standards set by Canadian Council on Animal Care and approved by the University of Lethbridge animal care committee.

Surgical Procedures

At 10 days of age all rats were anesthetized by cooling them in a Thermatron cooling chamber until their body temperatures were in the range of 18-20°C. Surgery was performed using suction by pipette. The surgeon used a surgical microscope to aid with the operation on these small animals (Kolb, Sutherland, & Whishaw, 1983). Rats received either a complete left hemisphere decortication or a scalp incision. The pups were then warmed up by hand and placed on a heating pad until they were all moving. All pups were then returned to the dam.

Treatment Procedures

Half of the lesion group and half of the sham group received subcutaneous injections of 10μg/kg of FGF-2. The treatment began the day after surgery and was given daily for one week.

Human recombinant FGF-2 (R&D Systems, Minneapolis MN: #233-FB) was dissolved in a phosphate buffer solution containing 1mg/ml of bovine serum albumin.

Food restriction

Subjects were kept on a restricted food regime. Each animal received 20g of food per day after the testing session was complete. Their body weight was maintained at ≈ 90-95% until the completion of the behavioural testing.

Morris Water Task

The maze consists of a circular pool (diameter 1.5 m, height 45 cm), the inside of which was painted white and filled with water at about 24°C mixed with instant powdered milk. A clear Plexiglas platform (11cm x12 cm) was hidden inside the pool (Kolb et al., 1983). (See illustration, Figure 1-5)

One trial consisted of placing the rat into the pool, facing the wall of the pool, at one of four starting locations. Four trials per day were carried out, each trial beginning at a different starting location. Each day the order of the starting locations was altered.

A computer tracking system measured the distance swum by the rat from the starting location until it reached the platform.

Food restriction

Subjects were kept on a restricted food regime. Each animal received 20g of food per day after the testing session was complete. Their body weight was maintained at ≈ 90-95% until the completion of the behavioural testing.

Tray reaching

The animals were placed in a test cage (10x18x10 cm high) with floor and fronts constructed of 2mm bars, 9 mm apart edge to edge. A 4 cm wide and 1 cm deep tray, containing chicken feed pellets, was mounted in the front of each box (Figure 1-6).

The rats were food deprived to 90% of their normal body weight for testing. The rats were required to extend a forelimb through the gap in the bars, grasp and retract the food and eat it. Animals were tested every day for three weeks in the tray reaching apparatus. They were allowed to reach with either paw. At the end of each test week, rats were filmed for 5 mins and the tapes were scored for numbers of attempted and successful reaches. The percent success was calculated as follows:

(Successful reaches/ (successful reaches + attempts)) x 100%

This was calculated for each week and then averaged.

Single Pellet reaching

The single pellet reaching boxes were made out of clear Plexiglas with the dimensions 45cm deep x 14cm wide x 35cm high. In the centre of the front wall was a 1 cm wide slit which extended from 2 cm above the floor to 15 cm above (see Figure 1-7). In front on the slit on the outside of the box there was a 2 cm wide shelf. The shelf was placed 3 cm above the floor. Two indentations were made on the shelf 2 cm from the inside of the wall. The indentations were centred to the slit so the rats could easily reach them. Food pellets (45 mg Rodent Chow food pellets, Bioserve) were placed in the indentation opposite to the rats' preferred reaching paw.

The animals were trained as adults for 2 weeks and then tested for nine days on the single pellet reaching task. They were presented with 20 pellets in each testing session. A successful reach was defined as the pellet being retrieved from the platform and eaten by the animal. At times the rat would reach and miss the pellet but repeat the reaching movement until it finally retrieved the pellet. Each reaching movement was counted as an attempt. The performance of each animal was analyzed in two ways. First, the number of successful reaches out of twenty trials was analyzed. Second, the accuracy of each reach was analysed. This was calculated as follows:

Number of attempts/ # of pellet successfully retrieved

An attempt was classified as the motion of reaching for the pellet. A score of 1 is perfect, as it would take one attempt to retrieve one pellet.

Forepaw Asymmetry

Forelimb use during spontaneous exploration was examined by placing rats in a transparent cylinder 20 cm in diameter and 30 cm in height (Schallert, Kozlowski,

Humm, & Cocke, 1997). A mirror was placed beneath the cylinder to allow the activity of the rat to be videotaped. For the video analysis, each forepaw contact with the cylinder wall was counted (Figure 1-8). The asymmetry score [i.e. contralateral forelimb/ (contralateral + ipsilateral)] of forepaw use was calculated for both the first touch of any bout of exploration and the total amount of touches for the testing period. Animals were individually placed in the cylinder for a single, three minute testing session.

Sunflower Seed Task

Five sunflowers seeds were placed in the corner of a clear Plexiglas box (50 x 50 x 50cm). The rat was allowed to explore the box until it discovered the seeds. Rats began by manipulating the seeds into their preferred position before removing the shell (Figure 1-8). The animal would then eat all five seeds in succession. The total amount of time which the animal spent manipulating, opening, and consuming the seeds was recorded. Animals were trained for one day and tested on the second and third day.

Anatomical Procedures

The animals were separated by sex for the anatomical studies. Only male brains were stained with Golgi-Cox and only female brains were mapped with ICMS.

Male animals were given an overdose of sodium pentobarbital and perfused intracardially with 0.9% saline. The brains were removed and weighed before being submerged in ~20mL of Golgi-Cox solution. The brains were left in this condition for 14 days and then placed in a 30% sucrose solution for two days. The brains were cut on a vibratome at 200μm and developed using the procedure described by Gibb & Kolb (1998). Layer III pyramidal cells were traced in Zilles' parietal area 1 using camera lucida at 250X magnification. Dendritic length and branching were measured on these

drawings. Branch order analysis was done according to Coleman & Riesen (1968). Branch length also was measured indirectly by Sholl analysis (1956).

Cortical Thickness

Cortical thickness was measured on the Golgi-Cox stained brains. Only males were used for this analysis. The dimensions were taken on a Zeiss 2 POL projector set at a magnification of 10x. Measurements were taken at three different points at each of five planes corresponding to Zilles' levels +2.2, -0.3, -2.3, -4.8, and –6.3 relative to bregma, (Stewart & Kolb, 1988). The planes were chosen for their correspondence to the anterior tip of the caudate-putamen, the middle of the anterior commisure, the rostral tip of Ammon's horn, the posterior commisure, and the posterior tip of the hippocampus. The multiple measurements from each plane were averaged and then all five planes were averaged again.

Statistical Analysis

Analyses of variance (ANOVA) were used for all measurements and Fisher's LSDs ($p < 0.05$ or less) were used for *post hoc* evaluations. Because no sex differences could be measured the data were collapsed across this factor.

BEHAVIOURAL RESULTS

Morris Water Task

Rats with hemidecortications were impaired at this task [F (1,51) = 43.133, $p<$ 0.0001] and FGF-2 injections enhanced this impairment (Figure 4-1A). There was a main effect of the FGF-2 injections [F (1,51) = 13.135, $p < 0.01$]. ANOVA also demonstrated a Lesion x Treatment interaction [F (1,51) = 4.955, $p < 0.05$]. Normal rats hold their forelimbs motionless when they swim in open water, a behaviour that allows faster travel through the water. In contrast, hemidecorticate rats paddle with the forepaw contralateral to the lesion, which slows down swimming speed (e.g., Kolb & Tomie, 1988). Swim distance was therefore used as the measure for statistical analysis. A repeated measures ANOVA displayed an effect of day [F (4,204) = 33.347, $p< 0.0001$] (Figure 4-1B). There was also a significant Day x Lesion interaction [F (4,204) = 32.907, $p< 0.05$]. A Fisher's LSD revealed a significant difference between the overall average of the two lesion groups ($p< 0.001$).

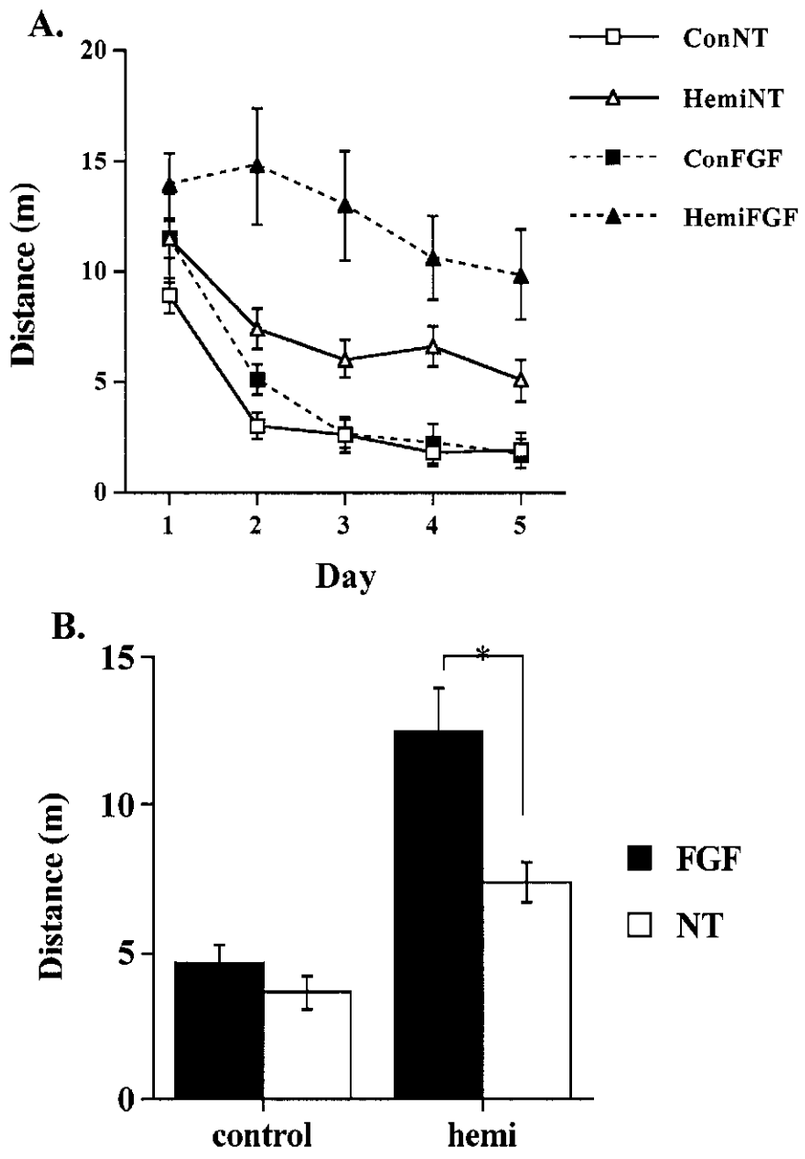

Figure 4-1. Morris water task. Distance was measured in the Morris water task because the hemidecorticated animals displayed some motor impairments which affects time measurement. The hemidecorticate animals were impaired at this task. The treatment further impaired the injured animals. A. Average daily swim distance. B. Overall average distance over the five days. Hemi; P 10 hemidecorticate, FGF; Fibroblast Growth Factor-2, NT; no treatment. (* < 0.05)

Tray Reaching

The hemidecorticate rats all reached with their limb ipsilateral to the lesion. Nonetheless, the hemidecorticate groups were impaired [F (1,61) = 13.657, $p < 0.01$] and FGF-2 had no significant effect on performance [F (1,61) = 0.571 p=0.4529] (Figure 4-2). ANOVA showed no Lesion x Treatment interaction [F (1,61) = 0.057, $p = 0.8114$].

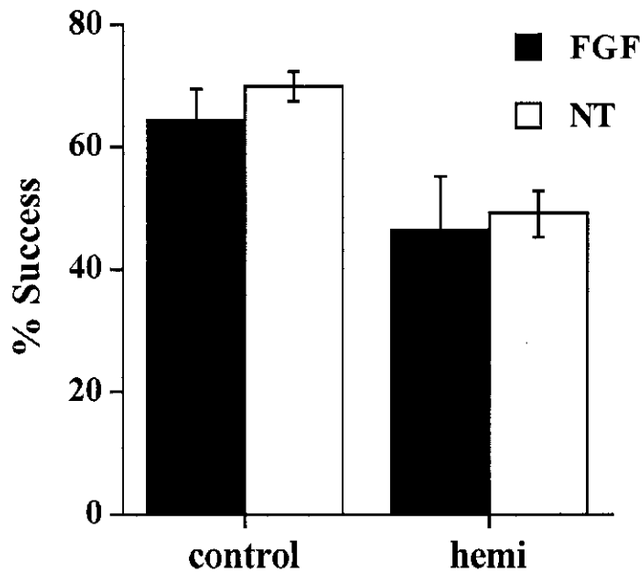

Figure 4-2. Tray Reaching. Reaching success was measured over three weeks. The hemidecorticate animals were impaired at this task when compared to controls. The treatment had no effect on either group. Hemi; P 10 hemidecorticate, FGF; Fibroblast Growth Factor-2; NT; no treatment.

Single Pellet Reaching Task

As in the tray reaching task, all hemidecorticate animals used their paw ipsilateral to the damaged hemisphere when reaching. The hemidecorticate groups were significantly impaired at retrieving pellets in the single pellet reaching task [$F(1,38) = 16.800$, $p < 0.01$] and thus retrieved fewer pellets per session (Figure 4-3A). As discussed in chapter two of this thesis, the injured animals displayed mirror movements with their forepaws (Figure 2-2). There was no effect of the FGF-2 treatment on the number of pellets retrieved [$F(1,38) = 1.682$, $p = 0.2025$]. There was no Lesion x Treatment interaction [$F(1,38) = 0.206$, $p = 0.6526$] for the number of pellets retrieved.

Figure 4-3B is a reflection of the accuracy of the animals. The graph displays the average number of reaches/pellet that was successfully retrieved. A two-way ANOVA showed that the hemidecorticated groups were not significantly different [$F(1,38) = 0.576$, $p = 0.4526$] and the treatment had no main effect on this measure [$F(1,38) = 0.228$, $p = 0.6361$]. There was also no Lesion x Treatment interaction [$F(1,38) = 0.082$, $p = 0.7758$].

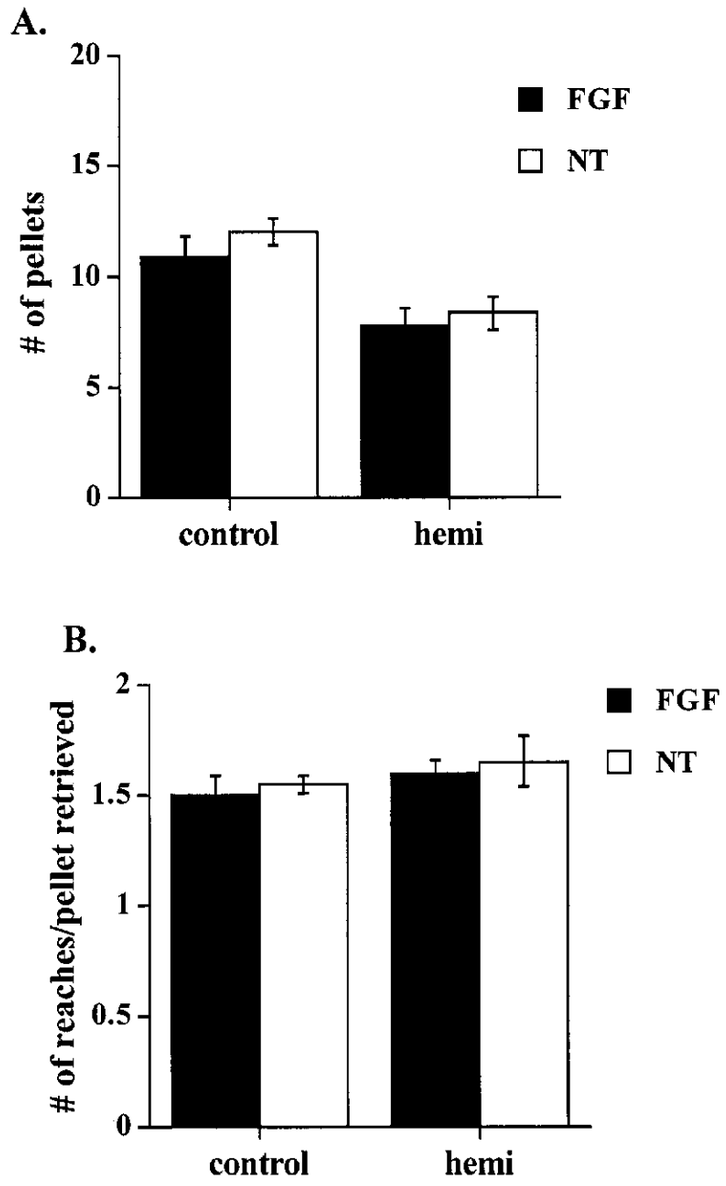

Figure 4-3. Single pellet reaching. The hemidecorticate animals were impaired at retrieving pellets but showed no difference in accuracy from controls. There was no effect of treatment on either measure. A. Number of pellets successfully retrieved. B. Accuracy of the animals retrieving the pellets. Hemi; P 10 hemidecorticate, FGF; Fibroblast Growth Factor-2, NT; no treatment.

Forepaw Asymmetry

The hemidecorticate animals relied significantly more on the paw that was ipsilateral to the damaged hemisphere for support in the cylindrical apparatus [F (1,51) = 26.843, $p < 0.0001$] and there was no benefit of FGF treatment [F(1,51) = 0.088, $p = 0.7674$]. There was a significant Lesion x Treatment interaction, however, [F (1,51) = 5.447, $p < 0.05$]. The interaction reflected the relative FGF-2 effects in the control and hemidecorticate groups as seen in Figure 4-4. These effects were small although there was a significant difference between the two control groups ($p< 0.05$).

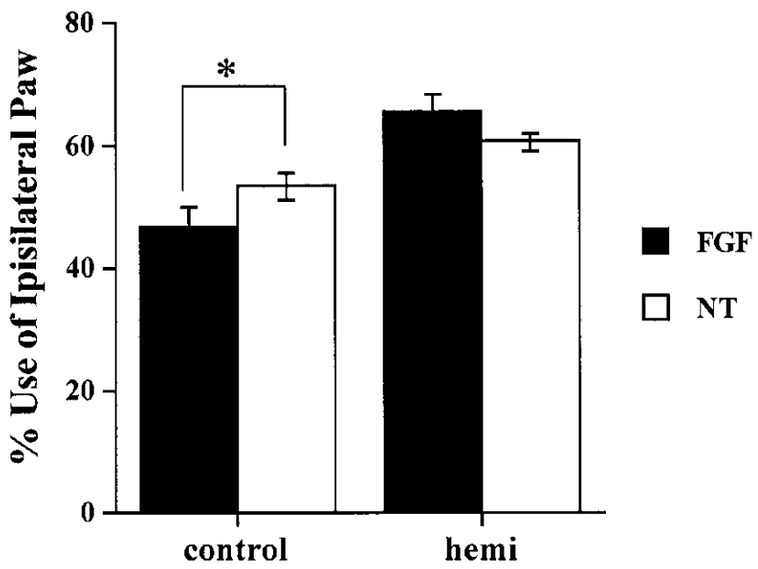

Figure 4-4. Forepaw asymmetry of paw use in exploring a novel environment. The hemidecorticate animals relied more on their ipsilateral-to-injury paw. There was an effect of the treatment on the control animals. Hemi; P 10 hemidecorticate, FGF; Fibroblast Growth Factor-2, NT; no treatment. (* < 0.05)

Sunflower Seed Consumption Task

There was a significant difference between the groups at consuming five sunflower seeds [F (1,48) = 15.092, p <0.01]. The hemidecorticate animals took an average of 20 seconds longer to open the seeds than control animals (Figure 4-5). There was no effect of the FGF-2 on the performance of this task [F (1,48) = 0.956, p = 0.3331]. No Lesion x Treatment interaction was noted [F (1,48) = 0.227, p = 0.6363]. A Fisher's LSD showed that the hemidecorticate groups differed from their respective control groups (p <0.05 or better).

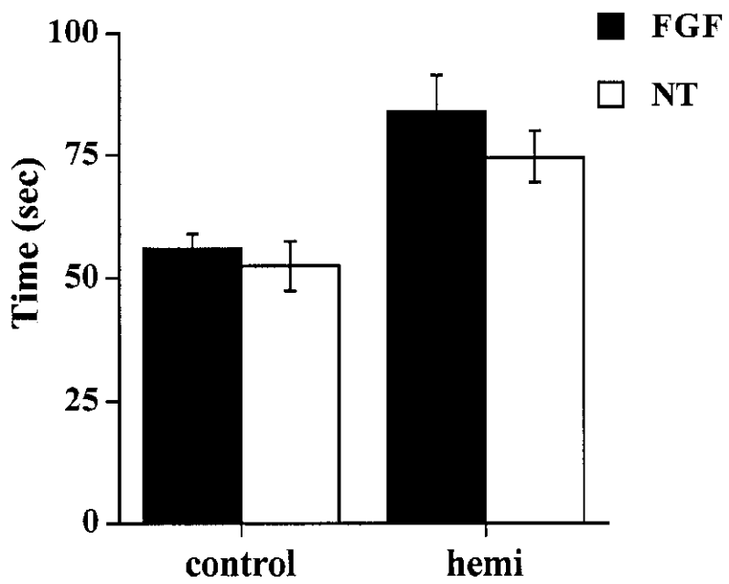

Figure 4-5. Sunflower Seed Consumption Task. Time, in seconds, to consume five sunflower seeds. The hemidecorticate animals were significantly impaired at opening and eating the seeds. Hemi; P 10 hemidecorticate, FGF; Fibroblast Growth Factor-2, NT; no treatment.

ANATOMICAL RESULTS

After cardial perfusion, a visual inspection was made of the brains. There were no obvious visual differences due to the treatment in either the control or injured group (Figure 4-6).

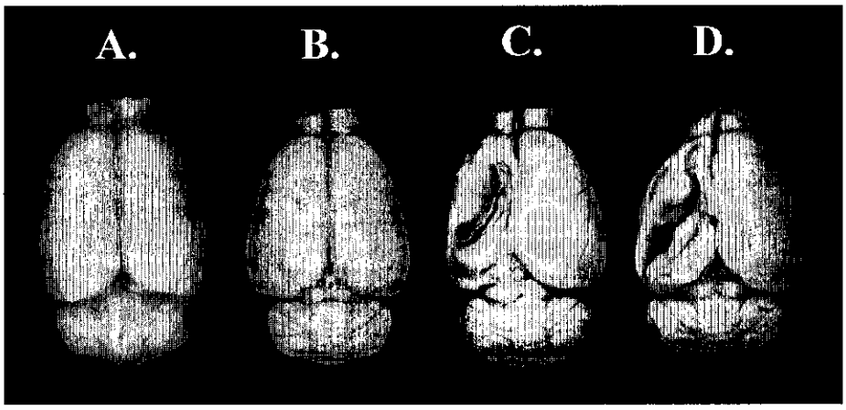

Figure 4-6. Male brains after perfusion and two weeks in Golgi-Cox solution. A; control with FGF-2 injection, B; control, C; P 10 hemidecorticate with FGF-2 injection, D; P 10 hemidecorticate.

Male Brain Weight

The brains of the male animals were weighed and compared after perfusion. The hemidecorticate animals had large lesions and this was reflected in the significant weight difference between the hemidecorticate and control animals [$F(1,38) = 37.720$, $p < 0.0001$]. There was no effect of the FGF-2 injections on brain weight [$F(1,38) = 0.859$, $p = 0.3599$]. There was also no Lesion x Treatment interaction [$F(1,38) = 0.122$, $p = 0.7292$].

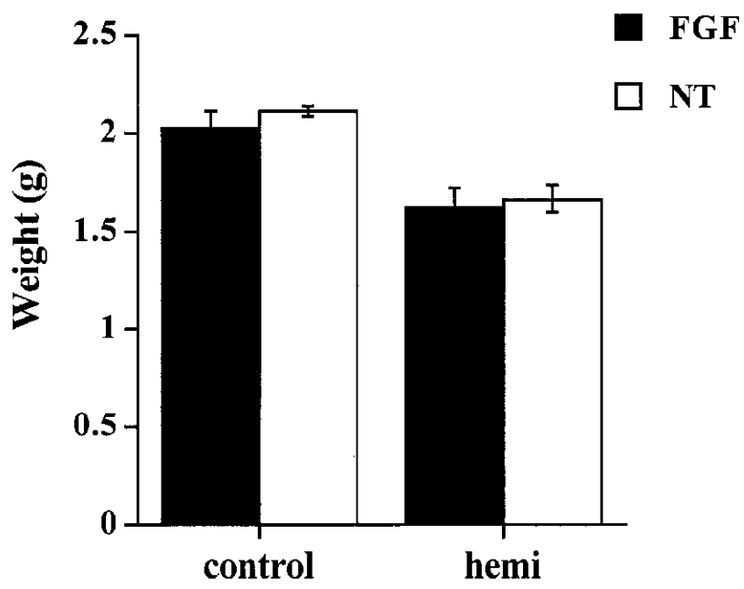

Figure 4-7. Average brain weights of male rats. There was a significant effect of the hemidecortication but no effect of treatment on either group. Hemi; P 10 hemidecorticate, FGF; Fibroblast Growth Factor-2, NT; no treatment.

Cortical Thickness

Cortical thickness was measured throughout the intact hemispheres and a comparison of mean cortical thickness showed no main effect of lesion [F (1,12) = 0.04, $p = 0.9886$] or treatment effects [F (1,12) = 0.958, $p = 0.3470$]. ANOVA showed no Lesion x Treatment interaction [F (1,12) = 0.007, $p = 0.9334$].

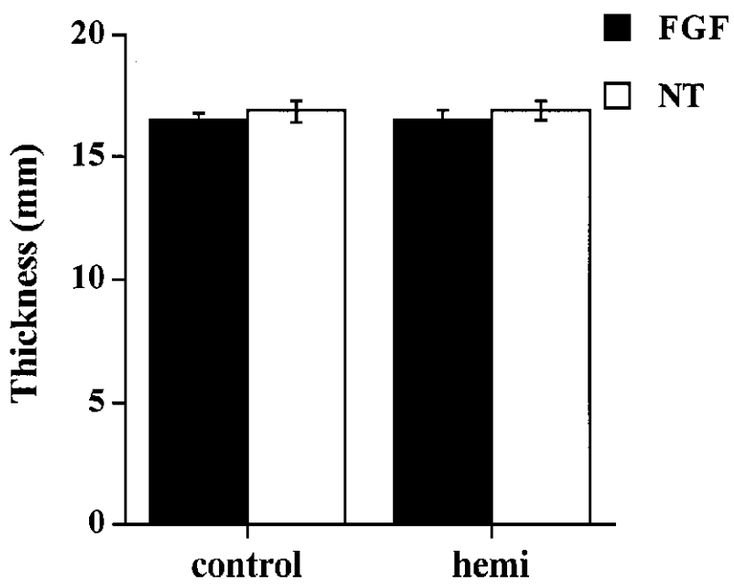

Figure 4-8. Cortical thickness of the remaining neocortex. There was no effect of the lesion or treatment on cortical thickness. Hemi; P 10 hemidecorticate, FGF; Fibroblast Growth Factor-2, NT; no treatment.

Golgi-Cox Analysis

Cells were drawn from the hemisphere contralateral to injury in the lesion animals and in both hemispheres of the control animals. As shown in Figures 4-9 and 4-10, neither the lesion nor the FGF-2 treatment had significant effects on the complexity or length of dendritic branching in layer III parietal cells.

The first two graphs (Figure 4-9) show the measurements taken for the apical branches. A two-way ANOVA on the apical fields showed no main effect of lesion [F (1,14) = 0.467, p = 0.5056], treatment [F (1,14) = 1.358, p = 0.2633], nor a Lesion x Treatment interaction [F (1,14) = 0.504, p = 0.4894] for the branch order analysis (Figure 4-9A). There was also no main effect of lesion [F (1,14) = 1.632, p = 0.2222], treatment [F (1,14) = 0.204, p = 0.6586], nor Lesion x Treatment interaction [F (1,14) = 0.140, p = 0.7135] for dendritic length (Figure 4-9B).

. The bottom two graphs (Figure 4-10) display the measurements from the basilar tree. A two-way ANOVA on the branch order for the basilar fields showed no significant main effect of lesion [F (1,14) = 0.148, p = 0.7062], FGF-2 treatment [F (1,14) = 0.592, p = 0.4544], nor Lesion x Treatment interaction [F (1,14) = 3.105, p = 0.0998] (Figure 4-10A). There was again no main effect of lesion [F (1,14) = 0.135, p = 0.7191], treatment [F (1,14) = 0.685, p = 0.4217], nor Lesion x Treatment interaction [F (1,14) = 0.154, p = 0.7006] for the basilar dendritic length (figure 4-10B).

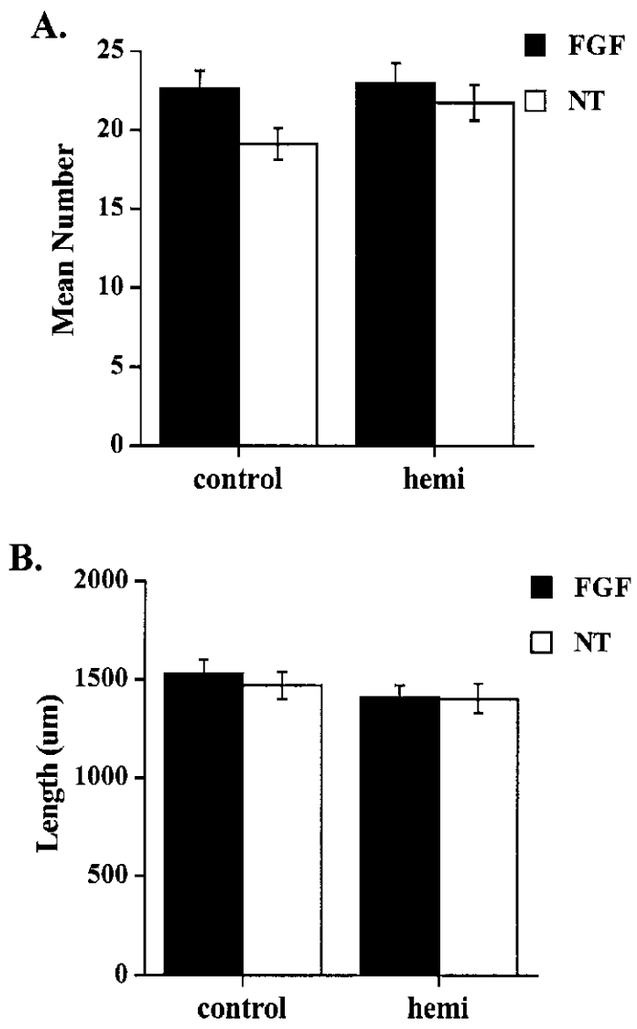

Figure 4-9. Measurements taken from the apical dendrites of parietal layer III cells. Cells were drawn on in the contralateral side of the hemidecorticate animals. There was no effect of lesion or treatment on the apical branches. A. Dendritic complexity (branch order). B. Dendritic length. Hemi; P 10 hemidecorticate, FGF; Fibroblast Growth Factor-2, NT; no treatment.

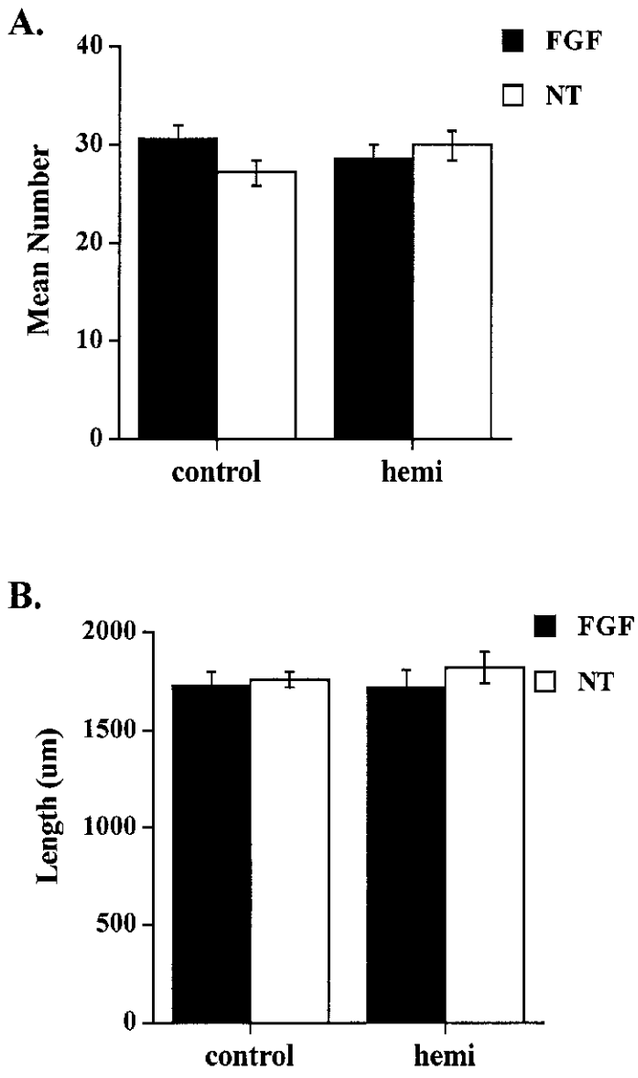

Figure 4-10. Measurements taken from the basilar dendrites of parietal layer III cells. Cells were drawn on in the contralateral side of the hemidecorticate animals. There was no effect of lesion or treatment on the basilar branches. A. Dendritic complexity (branch order). B. Dendritic length. Hemi; P 10 hemidecorticate, FGF; Fibroblast Growth Factor-2, NT; no treatment.

DISCUSSION

Rats that receive postnatal day ten hemidecortication show impaired motor and cognitive abilities. This has been reported by Kolb and Tomie (1988) and in previous experiments in this thesis. Injections of FGF-2 did not improve or hinder the motor skills of the hemidecorticate animals. This treatment did however further impair the injured rats in the Morris water task.

Hastings has shown that FGF-2 improves functional outcome after postnatal day three (P 3) bilateral frontal lesions (Hastings, 2003). Hastings used a similar battery of tests but both motor and cognitive improvements were noted. There are obvious differences between the two experiments, however, such as type of lesion and the day on which it occurred.

Monfils *et al.* (2005) has demonstrated that FGF-2 injections after bilateral postnatal day ten lesions improve functional recovery. Again, the lesions are different but the day of injury is the same. The major difference therefore lies in the type of lesion.

In the second week of life (P 7 –12) in a rat, cells called astrocytes begin to function (Kolb, 1995). These cells are known to surround areas of cortical damage and produce FGF-2. It is also known that bilateral medial frontal lesions that are administered at this time show impressive functional recovery. These frontal lesions even show a regeneration of tissue in the lesion site. This response to medial frontal injury was not seen after P 10 hemidecortication. The decorticated hemisphere showed no signs of regeneration of the neocortex.

One possibility for this difference between the two types of lesions is that qualities of the remaining tissue, surrounding the prefrontal focal lesions, are responsible

for the regeneration. A second possibility is that because of the complete loss of neocortical tissue, the brain is not able to allocate growth factors and other neurotropic aids that might facilitate the regeneration of tissue in the P 10 medial frontal lesions. A final possibility is that the contralateral hemisphere mediates the recovery in the focal lesion. However, because no regeneration of tissue could be noted in the hemidecorticate animals, this does not seem likely.

The brain clearly has a different natural response to focal lesions and hemidecortication at postnatal day ten. It is therefore not as surprising that the response to injections of FGF-2 is not the same. Because the initial lesion creates a different brain, the treatments most likely do not function in the same manner.

In the previous chapter it was noted that tactile stimulation improves performance in the Morris water task (Figure 3-1). One theory for this improvement is that tactile stimulation decreases glucocorticoids (a stress hormone) throughout a rat's life (Meaney, Aitken, van Berkel, Bhatnagar, & Sapolsky, 1988). Gibb has previously shown that tactile stimulation of rats increases FGF-2 in the brain and skin (Gibb, 2001). Tactile stimulation is a non-invasive treatment, however. At times, animals have been known to sleep or groom during the administration. This is not the case for administration of FGF-2. This treatment requires the animals to be injected subcutaneously for one week. Even though extremely care is taken not to harm any of the neonates, it is a stressful experience. It may be that the stress of the administration early in development alters the cognitive abilities of these animals later in life. Further studies will have to measure glucocorticoid levels in adulthood to investigate this theory.

Injections of FGF-2 clearly offer different outcomes based on the type of injury that the rat has sustained. The current study demonstrates that FGF-2 is not effective treatment after P 10 hemidecortication.

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5.GENERAL DISCUSSION

Two goals were stated at the beginning of this thesis: (1) to determine the behavioural effects of hemidecortication in neonatal and adult rats; and, (2) to explore the effectiveness of two treatments on functional recovery after neonatal hemidecortication. In three separate experiments, rats were hemidecorticated and tested on a battery of motor tasks. Furthermore, anatomical changes were examined at the cellular level in the neocortex contralateral to the insult.

The experiments from this thesis detailed several important findings. First, rats that received neonatal hemidecortication displayed mirror movements of the forelimbs. Second, adult and neonatal hemidecortication produced different anatomical changes in the contralateral neocortex. Third, hemidecortication did not display the same regeneration of tissue as small focal lesion. Forth, tactile stimulation produced mild improvement of functional outcome in rats after neonatal hemidecortication. Fifth, injections of FGF-2 further impaired cognitive function after neonatal hemidecortication.

Rats that receive neonatal hemidecortication displayed forelimb mirror movements.

Rats with P 10 lesions reached for single food pellets with their ipsilateral-to-lesion forelimb. Instead of relying on the contralateral-to-lesion forelimb for postural support, it appeared to perform a reach-like movement in conjunction with the ipsilateral-to-lesion forelimb. Interestingly, such reaches were not directed towards the pellet, as the forelimb often did not advance through the slot in the apparatus. Thus, the contralateral-to-lesion forelimb mirrored the reaching movements of the ipsilateral-to-lesion forelimb (Figure 2-2). A similar behaviour was coined a mirror movement by Woods & Teuber (1978). Their study used human subjects and showed that the mirror movements could

occur at varying degrees depending on when the injury occurred. This is one of the first times this behaviour has been noted in an awake, behaving rat.

Castro *et al.* (1986) evoked bilateral forelimb movements with electrical stimulation of one hemisphere in anaesthetized animals. The lesions in Castro's studies included prefrontal, frontal and motor cortex. All neocortex posterior to this was spared. Thus, the brain that they were working with most likely had different connections.

There are two possible mechanisms for the altered formation that supports the mirror movements. First, because the injury occurred during a phase of development that supports axon growth, cortico-spinal projections may have formed between the intact neocortex and motoneurons controlling the ipsilateral-to-lesion forelimb. Such projections develop naturally in rats albeit sparsely. In this case, motor cortex signals from the intact hemisphere that normally drive the contralateral forelimb are likely to also drive the ipsilateral forelimb. It would therefore appear as though the ipsilateral and contralateral forelimbs are copying one another. A second possibility is that the lesion interferes with normal development of the intact hemisphere. At birth, cortico-spinal projections originate from multiple neocortical areas and project densely to both sides of the spinal cord. Pruning of such connections produces the adult-like cortico-spinal projection, which primarily arise in motor cortex and project contralaterally to the spinal cord. If the lesion interrupts this pruning process, projections from the intact neocortex would be equally distributed to both sides of the spinal cord and drive both forelimbs simultaneously.

Adult and neonatal hemidecortication produce different anatomical changes in contralateral neocortex.

The first difference between P 10 and P 90 hemidecorticate rats is that adult hemidecorticates had thinner neocortex throughout the brain whereas there was no change in cortical thickness in the P 10 animals. The loss of thickness in the adult animals presumably reflects the loss of cortico-spinal connections. It is surprising that the absence of these connections did not alter cortical thickness in the P 10 animals, suggesting that the layer III cells that normally form the callosal projections had established other, anomalous, connections.

The second difference is that P 90 hemidecorticate animals also had much larger, more complex layer III pyramidal cells in the parietal cortex in the contralateral hemisphere than both P 10 hemidecorticates and control animals. The hypertrophy of cells is one way that the brain can change after damage. This is one way cells can compensate for the loss of projections from the contralateral neocortex. Again, the increase in dendritic complexity, combined with the loss of cortical thickness in the adult hemidecorticates is surprising. The Golgi technique did not allow us to determine the connections of the neurons drawn but we can speculate that these neurons had cortico-cortical connections and the collosally-projecting neurons may have died. This would account for the decreased cortical thickness combined with the increased dendritic fields.

Hemidecortication did not display the same regeneration as small focal lesion at postnatal day ten.

Previous studies by Kolb and Gibb (Kolb, 1987; Kolb & Gibb, 1993) have shown that if medial prefrontal cortex is removed at postnatal day ten there is a regeneration of

tissue in the lesion site. This was not seen with hemidecortication at P 10. One possibility for this difference between the two types of lesions is that qualities of the remaining tissue surrounding the prefrontal focal lesions are responsible for the regeneration. A second possibility is that because of the complete loss of neocortical tissue, the brain is not able to allocate growth factors and other neurotropic aids that might facilitate the regeneration of tissue in the P 10 medial frontal lesions. A final possibility is that the contralateral hemisphere mediates the regeneration in the contralateral hemisphre after a focal lesion. However, because no regeneration of tissue could be noted in the hemidecorticate animals, this does not seem likely.

Future studies that wish to address this question might examine P 10 hemidecortication and simultaneous medial frontal lesion on the contralateral hemisphere. If the medial frontal area regenerates it can be shown that the contralateral hemisphere plays no role in the process.

Tactile stimulation produced mild improvement of functional outcome in rats after neonatal hemidecortication.

Neonatal hemidecorticate animals received a week of tactile stimulation treatment three times a day for 15 minutes. This treatment improved the performance of animals in the tray reaching task, the sunflower seed consumption task, and the Morris water task. There are two possibilities for these improvements.

First, tactile stimulation may strengthen the connections that are responsible for the mirror movements. Both of the motor tasks that showed improvement due to the tactile stimulation treatment allowed a freedom of movement so that the rats could use both forepaws at the same time. This allowed treatment induced improvements to be

measured. The single pellet reaching task limited the movement of one paw, therefore it was impossible to measure any improvement due to the treatment.

Second, the behavioural improvements may be due to a decrease in stress. Previous studies have demonstrated that neonatal handling reduced stress and age related impairments associated with the hippocampus (Meaney, Aitken, van Berkel, Bhatnagar, & Sapolsky, 1988). This group showed that handling rats decreased levels of glucocorticoids (a stress hormone) at all ages of life. Stress can interfere with both cognitive and motor tasks. Further studies will have to be done to measure glucocorticoid levels.

This notable improvement in P 10 hemidecorticate animals after tactile stimulation raises questions about how rats are housed. As mentioned before, when rats are housed in large groups, the animals receive an increased amount of natural tactile stimulation. Perhaps the greater functional recovery is due to a more normal environment. This would mean that the "impoverished" standard housing that animals' are normally reared in is responsible for the poor recovery in the non-treated hemidecorticate rats.

To test this hypothesis, rats could be bread and housed in an enriched environment to see if this furthers the improvement in hemidecorticated animals that was noted after tactile stimulation. This would also allow for another comparison between focal and large neocortial lesions.

Injections of FGF-2 further impaired cognitive outcome after neonatal hemidecortication.

Animals that received P 10 hemidecortication and post-surgical injections of FGF-2 showed impaired cognitive spatial abilities. Neonatal hemidecortication hinders the cognitive abilities of rats (Kolb & Tomie, 1988). The injections of FGF-2 further impaired these animals in the Morris water task. There are two likely explanations for the FGF-2-induced impairments.

First, the administration of the FGF-2 required animals to receive subcutaneous injections for one week (from postnatal day 11 to postnatal day 17). It may be that the stress of the administration, early in development, in correlation with the large cortical lesion alters the cognitive abilities of these animals later in life. There are two possibilities for increased stress during the injections. (1) The injection process requires the infantile rat to be held firmly in the hand of the administrator. This is followed by an injection. This procedure is therefore one source of stress. (2) The pups were kept together in a carrying tub and injected one after the other. The cries from the sibling pups as they received the treatment may have increased the level of stress for these animals.

Second, there may be an optimal level of FGF-2 for an undamaged hemisphere. Adding FGF-2 thus may have raised the combined endogenous and exogenous FGF-2 to a toxic level. In contrast, when animals have a focal injury the increased FGF-2 in animals given FGF-2 injections may act on the injured hemisphere to enhance recovery. One piece of evidence in support of this idea is a study by Hastings (2003) who combined complex housing, which increases endogenous production of FGF-2, with FGF-2 injections in rats with day 3 frontal lesions. Both treatments alone were beneficial but the

combined treatment impaired recovery much as the FGF-2 injections did in the current study.

Limitations

There are two limitations in this thesis that need to be addressed. First, anterograde tracing techniques could have been used to support the mechanisms for the mirror movements. As it stands, there was no proof of the alterations in the cortico-spinal tract presented in this thesis. The spinal cords were not kept during the perfusions either. This prevented any further examination of the cortico-spinal tract.

Second, the tasks performed in this thesis were motor skill biased. One positive side of this is that novel behaviours, such as mirror movements, were observed because the motor tests chosen were sensitive and varied. The negative of relying heavily on motor tasks was that a complete analysis of the cognitive abilities of these hemidecorticate animals was not done. Time was a factor in completing this thesis however, and more work can be done in the future.

Practical Applications

Hemidecortication can affect both motor and cognitive abilities. Surgeons will perform this measure only in cases where epileptic activity cannot be controlled by any form of medication. It is not a procedure that is commonly practiced. Johns Hopkins Children's Center, for example, performs about 4 of these surgeries a year (Collins, 2002). The goal of research like that done in this thesis is to improve functional recovery in children who undergo this debilitating surgery. A treatment like tactile stimulation appears to have beneficial use for human children.

Tactile stimulation is an intelligent choice for several reasons: (1) The cost is low, although a human version of the blush brush used for the rats will have to be found. (2) There are no drug side effects. This method relies on the body's natural response to the stimulation.

This research also made an important point about when the surgery should take place. There is clearly a difference in recovery in neonatal and adult hemidecorticates. Although both groups were able to eat and move around their environment, it was the quality of movement on such tasks as single pellet reaching that was distinctly different. When deciding what age patients should undergo surgery both the quantity and quality of recovery of function must be considered.

Conclusions

There are two main conclusions from this project: (1) overall functional recovery is not better or worse but simply different based on the age at which the trauma occurred and (2) treatments have varied success with different types of brain injury.

As Margaret Kennard observed, animals recover differently based on the time of injury. What is not clear however is if she was correct in which stage of development offers the greatest resources for recovery of function. There were two patterns of functional compensation observed in the second chapter of this thesis. The neonatal (P 10) hemidecorticate animals displayed mirror movements. These can be beneficial for some tasks, such as the sunflower seed consumption task, but a hindrance in others, such as the single pellet reaching task. The adult hemidecorticate (P 90) animals however, lost all fine motor control of the paw contralateral to the injured hemisphere. This appeared to benefit the animals in the single pellet reaching task, but hindered the symmetry of the

animals when exploring a novel environment. The conclusion from these data is that overall functional recovery is not better or worse but simply different based on the age at which the trauma occurred.

With brain injury comes the desire to improve functional outcome. It is devastating to believe that nothing can be done to aid someone suffering after stroke or traumatic brain injury. Bryan Kolb and his colleagues have done much to improve the way treatments for brain injury are explored and understood. Simple treatments such as complex housing or tactile stimulation have proven to be extremely effective mechanisms for aiding in recovery after focal lesions. Applying these treatments to larger neocortical lesions does not show the same results. Although there were positive results with the tactile stimulation treatment, the FGF-2 injections displayed a detrimental effect after P 10 hemidecortication.

Future Directions

The future of this project is more anatomical work. There are three areas to investigate: (1) Analysing changes in protein levels by using a western blot technique; (2) Using tracers to observe the cortico-spinal and other cortico-subcortical connections that are present after hemidecortication; and (3) Perform an electrophysiological mapping technique that would allow measurements of sensori-motor connections.

The western blot technique could be used to analyse changes in proteins after hemidecortication. The first step would be harvesting cortical tissue from the hemisphere contralateral to the lesion. This study could examine changes in the remaining tissue at various times after the injury.

Previous studies with focal lesions have shown that growth factors such as FGF-2 are up regulated within the first week after frontal lesion (Gibb, 2004). To complete this body of work, western blot studies should be done after hemidecortication as well. Comparisons could then be done between different types of cortical injury. Understanding the brain's natural response to various injuries is an important part of developing treatments that are appropriate and effective.

A second project that requires investigation is tracing cortico-spinal connections. Evidence is required to establish that mirror movements were caused by bilateral projections of the cortico-spinal projections or possibly by anomalous cortico-subcortical connections. Tracers could be used in an anterograde and retrograde direction to properly establish exactly what connections are involved in these novel compensatory movements.

A final method that may allow for a more accurate understanding of the connections is electrophysiological mapping of sensory-motor connections. This technique allows for the stimulation to be done directly on the area of interest, such a paw, while neural activity is recorded in the cortex. This would compliment the intracortical microstimulation (ICMS) used in the second chapter of this thesis. Mirror movements could not be elicited during ICMS, but perhaps stimulating in the opposite direction will provide some concrete evidence of connections that have been established.

Hemidecortication in rats has been a model for studying brain function since the 1950's (Covian, 1952). Modern techniques in behavioural and anatomical analysis allow insights of how the brain reorganizes after large neocortical insult. By understanding what factors allow for better functional recovery following early brain injury it may be

possible to identify novel treatments and therapies for brain insult that occurs both later in life as well as during development.

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